



Production of desaturated fatty alcohols and desaturated fatty acyl acetates in yeast

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Publication date:
2018

Document Version
Publisher's PDF, also known as Version of record

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Citation (APA):
Borodina, I., Holkenbrink, C., Dam, M. I., Löfstedt, C., Ding, B., & Wang, H. (2018). Production of desaturated fatty alcohols and desaturated fatty acyl acetates in yeast. (Patent No. WO2018109167).

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(51) International Patent Classification:

C12N 9/02 (2006.01)

C12P 7/04 (2006.01)

(21) International Application Number:

PCT/EP20 17/083030

(22) International Filing Date:

15 December 2017 (15.12.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

16204783.1

16 December 2016 (16.12.2016) EP

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

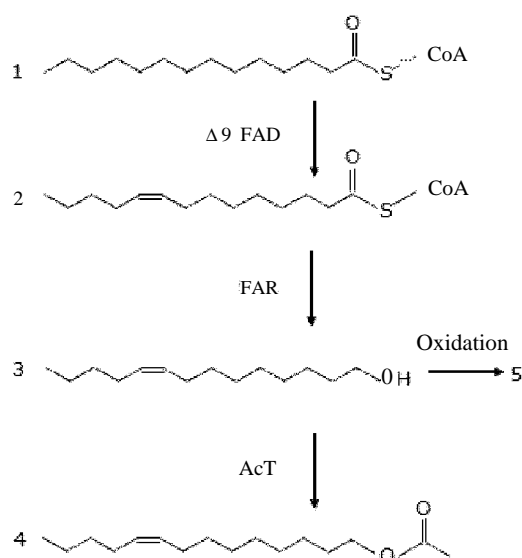
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— of inventorship (Rule 4.1 7(iv))

(54) Title: PRODUCTION OF DESATURATED FATTY ALCOHOLS AND DESATURATED FATTY ACYL ACETATES IN YEAST

FIG. 1



(57) Abstract: The present invention relates to the production of compounds comprised in pheromones, in particular moth pheromones, such as desaturated fatty alcohols and desaturated fatty acyl acetates and derivatives thereof, from a yeast cell.

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

Production of desaturated fatty alcohols and desaturated fatty acyl acetates in yeast

Technical field

The present invention relates to the production of compounds comprised in
5 pheromones, in particular moth pheromones, such as desaturated fatty alcohols and desaturated fatty acyl acetates and derivatives thereof, from a yeast cell.

Background

Since the advent of DDT more than 50 years ago, broad-spectrum neurotoxic
10 insecticides have provided the principal means for the control of economically important insects in agriculture and public health programs. Whereas the use of synthetic insecticides initially resulted in spectacular increases in crop yields and the suppression of some important human and animal disease vectors, the development of insecticide resistance in insect pest populations and the environmental damage caused
15 by insecticides have become widely recognized as serious drawbacks to their use. Among the most significant environmental problems associated with the manufacture and use of insecticides are 1) their direct toxicity to non-target organisms (including humans); 2) their persistence in the biosphere where they can accumulate and cause untoward developmental and reproductive effects in higher organisms; 3) significant
20 point-source pollution associated with their manufacture and distribution; 4) their worldwide dispersal.

Pheromones can be used as pest control instead of insecticides. (Z)1 9-14:OAc for
example has been found to disrupt mating efficiency of fall armyworm with 86%
25 efficiency when applied alone, i.e. without other pheromone components (Mitchell & McLaughlin, 1982). The commercial use of pheromones to control insect pests by mating disruption has several advantages over conventional insecticides. Pheromones are: 1) non-toxic and environmentally benign; 2) specific to one target species and do not adversely affect non-target beneficial insects, making them extremely well suited
30 for use in integrated pest management programs; and 3) much less likely (and have never been shown) to produce resistance in the target insect. In contrast to pheromone synthesis in nature, current approaches for the commercial production of pheromones employ traditional synthetic chemical routes. Because pheromones require very high

purity to elicit an insect's response, these synthesis methods are expensive and difficult, and generate large amounts of organic wastes that require treatment.

Thus the major hurdle standing in the way of using sex pheromones remains the production cost. As a result, a very small part of global agricultural land employs pheromones (estimated to less than 0.05%). Pheromone production from a cell factory is expected to significantly lower the production costs of pheromones.

Summary of invention

The invention is as defined in the claims.

Herein is provided a yeast cell capable of producing a desaturated fatty alcohol and optionally a desaturated fatty acyl acetate, said yeast cell expressing:

- i) at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA having a carbon chain length of 14; and
- ii) at least one heterologous fatty acyl-CoA reductase (FAR), capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol; and
- iii) optionally an acetyltransferase capable of converting at least part of said desaturated fatty alcohol to a desaturated fatty acyl acetate;

wherein the desaturase has a higher specificity towards tetradecanoyl-CoA than towards hexadecanoyl-CoA and/or wherein the fatty acyl-CoA reductase has a higher specificity towards desaturated tetradecanoyl-CoA than towards desaturated hexadecanoyl-CoA.

Also provided are methods for production of a desaturated fatty acid and optionally a desaturated fatty acyl acetate in a yeast cell, said method comprising the steps of providing a yeast cell and incubating said yeast cell in a medium, wherein the yeast cell expresses:

- i) at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA having a carbon chain length of 14, thereby converting at least part of said fatty acyl-CoA to a desaturated fatty acyl-CoA; and

- ii) at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, thereby producing said desaturated fatty alcohol; and
- iii) optionally an acetyltransferase capable of converting at least part of said desaturated fatty alcohol to a desaturated fatty acyl acetate, thereby producing said desaturated fatty acyl acetate;

wherein the desaturase has a higher specificity towards tetradecanoyl-CoA than towards hexadecanoyl-CoA and/or wherein the fatty acyl-CoA reductase has a higher specificity towards desaturated tetradecanoyl-CoA than towards desaturated hexadecanoyl-CoA.

Also provided herein are nucleic acid constructs for modifying a yeast cell, said constructs comprising:

- i) a first polynucleotide encoding at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA having a carbon chain length of 14; and
- ii) a second polynucleotide encoding at least one heterologous fatty acyl-CoA reductase (FAR), capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol; and
- iii) optionally a third polynucleotide encoding an acetyltransferase capable of converting at least part of said desaturated fatty alcohol to a desaturated fatty acyl acetate,

wherein optionally the first polynucleotide, the second polynucleotide and/or the third polynucleotide are under the control of a promoter.

Also provided is a kit of parts comprising:

- a) the yeast cell as disclosed herein and instructions for use; and/ or
- b) a nucleic acid construct as disclosed herein, wherein said construct is for modifying a yeast cell, and
- c) optionally the yeast cell to be modified.

Also provided is a desaturated fatty alcohol obtainable by the methods disclosed herein.

Also provided is a desaturated fatty acyl acetate obtainable by the methods disclosed herein.

Also provided is the use of a desaturated fatty alcohol as disclosed herein.

Also provided is the use of a desaturated fatty acyl acetate as disclosed herein.

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Description of the drawings

Figure 1: pathway towards Z9-C14:OAc. (1) tetradecanoyl-CoA (myristoyl-CoA), 14:CoA (2) (Z)9-tetradecen-1-yl-CoA, Z9-14:CoA, (3) (Z)9-tetradecen-1-ol, Z9-14:OH, (4) (Z)9-tetradecen-1-yl acetate, Z9-14:OAc, (5) (Z)9-tetradecenal, Z9-14:Ald. Δ^9 FAD - Z9-fatty acyl desaturase, FAR - fatty acyl-CoA reductase, AcT - acetyl-CoA transferase.

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Figure 2: expression cassettes. A: expression cassette of Dmd9 and HarFAR (encoded on plasmid pCfB6969). B: Expression cassette of Atf 1 (encoded on plasmid pCfB7600). C: Expression cassettes of *LIP2*, *LIP7*, or *LIP8*. "Term." Stands for terminator; "prom." Stands for promoter. D: Expression cassette of Atf 1 and Dmd9 (pCfB7235). E: Expression cassette of Dmd9 (pCfB7239). F: Expression cassette of SliDesl 1 (pCfB7240). G: Expression cassette of TesA(LL)/CcFATB1 and Dmd9 (pCfB7251/pCfB7253).

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Figure 3: Deletion of lipase genes in *Y. lipolytica* increases fatty alcohol titres.

Detailed description of the invention

Definitions

25 Biopesticide: the term 'biopesticide' is a contraction of 'biological pesticide' and refers to several types of pest management intervention: through predatory, parasitic, or chemical relationships. In the EU, biopesticides have been defined as "a form of pesticide based on micro-organisms or natural products". In the US, they are defined by the EPA as "including naturally occurring substances that control pests (biochemical pesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants containing added genetic material (plant-incorporated protectants) or PIPs". The present disclosure relates more particularly to biopesticides comprising natural products or naturally occurring substances. They are typically

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created by growing and concentrating naturally occurring organisms and/or their metabolites including bacteria and other microbes, fungi, nematodes, proteins, etc. They are often considered to be important components of integrated pest management (IPM) programmes, and have received much practical attention as substitutes to synthetic chemical plant protection products (PPPs). The Manual of Biocontrol Agents (2009: formerly the Biopesticide Manual) gives a review of the available biological insecticide (and other biology-based control) products.

Desaturated: the term "desaturated" will be herein used interchangeably with the term "unsaturated" and refers to a compound containing one or more double or triple carbon-carbon bonds.

Derived from: the term when referring to a polypeptide or a polynucleotide derived from an organism means that said polypeptide or polynucleotide is native to said organism.

Fatty acid: the term "fatty acid" refers to a carboxylic acid having a long aliphatic chain, i.e. an aliphatic chain between 4 and 28 carbon atoms, such as 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 carbon atoms. Most naturally occurring fatty acids are unbranched. They can be saturated, or desaturated.

Fatty acyl acetate: the term will herein be used interchangeably with "fatty acetate" and refers to an acetate having a fatty carbon chain, i.e. an aliphatic chain between 4 and 28 carbon atoms, such as 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 carbon atoms. Fatty acyl acetates can be saturated or desaturated.

Fatty acyl-CoA: the term will herein be used interchangeably with "fatty acyl-CoA ester", and refers to compounds of general formula $R\text{-CO-S-CoA}$, where R is a fatty carbon chain having a carbon chain length of 4 to 28 carbon atoms, such as 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 carbon atoms. The fatty carbon chain is joined to the -SH group of coenzyme A by a thioester bond. Fatty acyl-CoAs can be saturated or desaturated, depending on whether the fatty acid which it is derived from is saturated or desaturated.

Fatty alcohol: the term "fatty alcohol" refers herein to an alcohol derived from a fatty acyl-CoA, having a carbon chain length of 4 to 28 carbon atoms, such as 4, 5, 6, 7, 8,

9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 carbon atoms. Fatty alcohols can be saturated or desaturated.

5 Fatty aldehyde: the term refers herein to an aldehyde derived from a fatty acyl-CoA, having a carbon chain length of 4 to 28 carbon atoms, such as 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 or 28 carbon atoms. Fatty aldehydes can be saturated or desaturated.

10 Heterologous: the term "heterologous" when referring to a polypeptide, such as a protein or an enzyme, or to a polynucleotide, shall herein be construed to refer to a polypeptide or a polynucleotide which is not naturally present in a wild type cell. For example, the term "heterologous $\Delta 9$ desaturase" when applied to *Saccharomyces cerevisiae* refers to a $\Delta 9$ desaturase which is not naturally present in a wild type *S. cerevisiae* cell, e.g. a $\Delta 9$ desaturase derived from *Drosophila melanogaster*.

15 Native: the term "native" when referring to a polypeptide, such as a protein or an enzyme, or to a polynucleotide, shall herein be construed to refer to a polypeptide or a polynucleotide which is naturally present in a wild type cell.

20 Pest: as used herein, the term 'pest' shall refer to an organism, in particular an animal, detrimental to humans or human concerns, in particular in the context of agriculture or livestock production. A pest is any living organism which is invasive or prolific, detrimental, troublesome, noxious, destructive, a nuisance to either plants or animals, human or human concerns, livestock, human structures, wild ecosystems etc. The term
25 often overlaps with the related terms vermin, weed, plant and animal parasites and pathogens. It is possible for an organism to be a pest in one setting but beneficial, domesticated or acceptable in another.

30 Pheromone: pheromones are naturally occurring compounds. Lepidopteran pheromones are designated by an unbranched aliphatic chain (between 9 and 18 carbons, such as 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18 carbon atoms) ending in an alcohol, aldehyde or acetate functional group and containing up to 3 double bonds in the aliphatic backbone. Pheromone compositions may be produced chemically or biochemically, for example as described herein. Pheromones thus comprise
35 desaturated fatty alcohols, fatty aldehydes and/or fatty acyl acetates, such as can be obtained by the methods and cells described herein.

Saturated: the term "saturated" refers to a compound which is devoid of double or triple carbon-carbon bonds.

5 Specificity: the specificity of an enzyme towards a given substrate is the preference exhibited by this enzyme to catalyse a reaction starting from said substrate. In the present disclosure, a desaturase and/or a fatty acyl-CoA reductase having a higher specificity towards tetradecanoyl-CoA (myristoyl-CoA) than towards hexadecanoyl-CoA (palmitoyl-CoA) preferably catalyse a reaction with tetradecanoyl-CoA than with
10 hexadecanoyl-CoA as a substrate. Methods to determine the specificity of a desaturase or a fatty acyl-CoA reductase are known in the art. For example, specificity of a given desaturase can be determined by incubating cells that express said desaturase in a solution comprising methyl myristate for up to 48 hours, followed by extraction and esterification of the products with methanol. The profiles of the resulting
15 fatty acid methyl esters can then be determined by GC-MS. Desaturases with higher specificity towards myristoyl-CoA and low specificity towards palmitoyl-CoA will result in higher concentration of (Z)9-C14:Me than (Z)9-C16:Me. For example, specificity of a given reductase can be determined by incubating cells that express said reductase in a solution comprising methyl ester of (Z)9-myristate for up to 48 hours, followed by
20 extraction and analysis of the resulting fatty alcohols by GC-MS. Reductases with higher specificity towards (Z)9-C14:CoA and low specificity towards (Z)9-C16:CoA will result in higher concentration of (Z)9-C14:OH than (Z)9-C16:OH.

Desaturase

25 In the present disclosure, the terms 'fatty acyl-CoA desaturase', 'desaturase', 'fatty acyl desaturase' and 'FAD' will be used interchangeably. The term generally refers to an enzyme capable of introducing at least one double bond in E/Z conformations in an acyl-CoA having a chain length of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 carbon atoms. The double bond may be introduced in any position. For example, a
30 desaturase introducing a double bond in position 3 is termed Δ^3 desaturase. A desaturase introducing a double bond in position 5 is termed Δ^5 desaturase. A desaturase introducing a double bond in position 6 is termed Δ^6 desaturase. A desaturase introducing a double bond in position 7 is termed Δ^7 desaturase. A desaturase introducing a double bond in position 8 is termed Δ^8 desaturase. A
35 desaturase introducing a double bond in position 9 is termed Δ^9 desaturase. A

desaturase introducing a double bond in position 10 is termed $\Delta 10$ desaturase. A desaturase introducing a double bond in position 11 is termed $\Delta 11$ desaturase. A desaturase introducing a double bond in position 12 is termed $\Delta 12$ desaturase. A desaturase introducing a double bond in position 13 is termed $\Delta 13$ desaturase. A desaturase introducing a double bond in position 14 is termed $\Delta 14$ desaturase. A desaturase introducing a double bond in position 15 is termed $\Delta 15$ desaturase. A desaturase introducing a double bond in position 16 is termed $\Delta 16$ desaturase. A desaturase introducing a double bond in position 17 is termed $\Delta 17$ desaturase. A desaturase introducing a double bond in position 18 is termed $\Delta 18$ desaturase. A desaturase introducing a double bond in position 19 is termed $\Delta 19$ desaturase. A desaturase introducing a double bond in position 20 is termed $\Delta 20$ desaturase.

Desaturases catalyse the reaction (figure 1):

Fatty acyl-CoA + 2 ferrocytochrome b5 + O₂ + 2 H(+) \rightleftharpoons desaturated fatty acyl-CoA + 2 ferricytochrome b5 + 2 H₂O

For the purpose of the present disclosure, the desaturase is capable of introducing at least one double bond in a fatty acyl-CoA having a carbon chain length of 14.

The yeast cell disclosed herein expresses a desaturase having a higher specificity towards tetradecanoyl-CoA than towards hexadecanoyl-CoA and/or a acyl-CoA reductase having a higher specificity towards desaturated tetradecanoyl-CoA than towards desaturated hexadecanoyl-CoA. In other words, the desaturase is more specific for substrates having a carbon chain length of 14 than for substrates having a chain length of 16. Methods to determine the specificity of a desaturase or a fatty acyl-CoA reductase are known in the art. For example, specificity of a given desaturase can be determined by incubating cells that express said desaturase in a solution comprising methyl myristate for up to 48 hours, followed by extraction and esterification of the products with methanol. The profiles of the resulting fatty acid methyl esters can then be determined by GC-MS. Desaturases with higher specificity towards myristoyl-CoA and low specificity towards palmitoyl-CoA will result in higher concentration of (Z)9-C14:Me than (2)9-C16:Me. For example, specificity of a given reductase can be determined by incubating cells that express said reductase in a solution comprising methyl ester of (Z)9-myristate for up to 48 hours, followed by extraction and analysis of the resulting fatty alcohols by GC-MS. Reductases with higher specificity towards (Z)9-

C14:CoA and low specificity towards (Z)9-C16:CoA will result in higher concentration of (Z)9-C14:OH than (2)9-C16:OH.

5 In one embodiment, the cell is capable of expressing at least one heterologous $\Delta 5$ desaturase. In another embodiment, the cell is capable of expressing at least one heterologous $\Delta 6$ desaturase. In another embodiment, the cell is capable of expressing at least one heterologous $\Delta 7$ desaturase. In another embodiment, the cell is capable of expressing at least one heterologous $\Delta 8$ desaturase. In another embodiment, the cell is capable of expressing at least one heterologous $\Delta 9$ desaturase. In another
10 embodiment, the cell is capable of expressing at least one heterologous $\Delta 10$ desaturase. In another embodiment, the cell is capable of expressing at least one heterologous $\Delta 11$ desaturase. In another embodiment, the cell is capable of expressing at least one heterologous $\Delta 12$ desaturase. In another embodiment, the cell is capable of expressing at least one heterologous $\Delta 13$ desaturase. The gene encoding the
15 heterologous desaturase may be codon-optimised for the yeast cell, as is known in the art.

The skilled person will know, depending on which desaturated fatty alcohol is desired, which kind of desaturase to use. For example, for the production of a fatty alcohol
20 desaturated in position 11, a $\Delta 11$ desaturase is preferably used. If a fatty alcohol desaturated in position 9 is desired, a $\Delta 9$ desaturase may be used, such as a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 10 or a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Spodoptera litura* as set forth in SEQ ID NO: 12.

25 In one embodiment, the at least one heterologous desaturase is a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 10, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at
30 least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least
35 least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such

as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%,
such as at least 93%, such as at least 94%, such as at least 95%, such as at least
96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100%
homology to the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID
5 NO: 10.

In another embodiment, the at least one heterologous desaturase is a $\Delta 9$ desaturase
having at least 60% homology to the $\Delta 9$ desaturase from *Spodoptera litura* as set forth
in SEQ ID NO: 12, such as at least 61% homology, such as at least 62% homology,
10 such as at least 63% homology, such as at least 64% homology, such as at least 65%
homology, such as at least 66% homology, such as at least 67% homology, such as at
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15 at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such
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89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at
least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as
20 at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to
the $\Delta 9$ desaturase from *Spodoptera litura* as set forth in SEQ ID NO: 12.

In one embodiment, the heterologous desaturase is derived from *Pelargonium*
hortorum. In another embodiment, the heterologous desaturase is derived from
25 *Chauliognathus lugubris*. In some embodiments, the heterologous desaturase is
derived from *Drosophila melanogaster*.

The heterologous desaturase may be derived from an organism belonging to the order
of *Lepidoptera*. Thus in one embodiment, the heterologous desaturase is derived from
30 *Spodoptera litura*. In another embodiment, the heterologous desaturase is derived from
Choristoneura rosaceana. In another embodiment, the heterologous desaturase is
derived from *Choristoneura parallela*.

A heterologous desaturase may be expressed from a nucleic acid introduced in the
35 cell, e.g. on a vector such as a plasmid, or by genomic integration. The nucleic acid
may be codon-optimised as is known in the art for the specific yeast cell used.

In one embodiment, the at least one heterologous desaturase is encoded by a nucleic acid having at least 60% homology to the nucleic acid encoding the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 9, such as at least 61%
5 homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as
10 at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as
15 at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to the nucleic acid encoding the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 9.

In another embodiment, the at least one heterologous desaturase is encoded by a
20 nucleic acid having at least 60% homology to the nucleic acid encoding the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 36, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at
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30 as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to the nucleic acid encoding the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 36.

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In another embodiment, the at least one heterologous desaturase is encoded by a nucleic acid having at least 60% homology to the nucleic acid encoding the $\Delta 9$ desaturase from *Spodoptera litura* as set forth in SEQ ID NO: 11, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to the nucleic acid encoding the $\Delta 9$ desaturase from *Spodoptera litura* as set forth in SEQ ID NO: 11.

In some embodiments, the at least one heterologous desaturase is at least two heterologous desaturases, for example two heterologous desaturases. In some embodiments, the two heterologous desaturases are the $\Delta 9$ desaturase from *Drosophila melanogaster* Dmd9 and the $\Delta 11$ desaturase from *Amyelois transitella*, as set forth in SEQ ID NO: 10 and SEQ ID NO: 68, respectively.

The yeast cell to be modified may express a native desaturase, which may have a negative impact on the production of desaturated fatty alcohol and/or desaturated fatty acyl acetate. Accordingly, if the yeast cell to be modified expresses such a native desaturase, the cell may preferably be modified so that activity of the native desaturase is reduced or absent.

To ensure lack of activity of a native desaturase, methods known in the art can be employed. The gene encoding the native desaturase may be deleted or partly deleted in order to ensure that the native desaturase is not expressed. Alternatively, the gene may be mutated so that the native desaturase is expressed but lacks activity, e.g. by mutation of the catalytical site of the enzyme. Alternatively, translation of mRNA to an active protein may be prevented by methods such as silencing RNA or siRNA. Alternatively, the yeast cell may be incubated in a medium comprising an inhibitor

which inhibits activity of the native desaturase. A compound inhibiting transcription of the gene encoding the native desaturase may also be provided so that transcription is inactivated when said compound is present.

- 5 Inactivation of the native desaturase may thus be permanent or long-term, i.e. the modified yeast cell exhibits reduced or no activity of the native desaturase in a stable manner, or it may be transient, i.e. the modified yeast cell may exhibit activity of the native desaturase for periods of time, but this activity can be suppressed for other periods of time.

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Alcohol-forming fatty acyl-CoA reductase (EC 1.2. 1.84)

The terms 'alcohol-forming fatty acyl-CoA reductase', 'fatty acyl-CoA reductase' and 'FAR' will be used herein interchangeably. The term 'heterologous FAR' refers to a FAR which is not naturally expressed by the yeast cell.

15

FARs catalyse the two-step reaction (figure 1)

$\text{acyl-CoA} + 2 \text{ NADPH} \rightleftharpoons \text{CoA} + \text{alcohol} + 2 \text{ NADP}(+)$

wherein in a first step, the fatty acyl-CoA is reduced to a fatty aldehyde, before the fatty aldehyde is further reduced into a fatty alcohol in a second step. The fatty acyl-CoA
20 may be a desaturated fatty acyl-CoA.

The FARs capable of catalyzing such reaction are alcohol-forming fatty acyl-CoA reductases with an EC number 1.2.1 .84.

- 25 In some embodiments, the FAR is selected from the group consisting of Har_FAR (SEQ ID NO: 25, FAR from *Helicoverpa armigera*) or a variant thereof, such as the modified Har_FAR as set forth in SEQ ID NO: 27, Has_FAR (SEQ ID NO 29, FAR from *Helicoverpa assulta*) or a variant thereof, such as the modified Has_FAR as set forth in SEQ ID NO: 31, Hs_FAR (SEQ ID: 33, FAR from *Heliothis subflexa*) or a variant thereof, such as the modified Hs_FAR as set forth in SEQ ID NO: 35, and a Ban_FAR (SEQ ID NO: 45, FAR from *Bicyclus anynana*). In specific embodiments, the FAR is Har_FAR as set forth in SEQ ID NO: 25 or a variant thereof, such as the modified Har_FAR as set forth in SEQ ID NO: 27.
- 30

In one embodiment, the FAR is Har_FAR (SEQ ID NO: 25, FAR from *Helicoverpa armigera*) or a variant thereof having at least 75% homology to Har_FAR, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to Har_FAR (SEQ ID NO: 25).

In another embodiment, the FAR is a modified Har_FAR (SEQ ID NO: 27, FAR from *Helicoverpa armigera* wherein the signal peptide has been modified to HDEL) or a variant thereof having at least 75% homology thereto, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to the modified Har_FAR as set forth in SEQ ID NO: 27.

In another embodiment, the FAR is Has_FAR (SEQ ID NO: 29, FAR from *Helicoverpa assulta*) or a variant thereof having at least 75% homology to Has_FAR, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to Has_FAR (SEQ ID NO: 29).

In another embodiment, the FAR is a modified Has_FAR (SEQ ID NO: 31, FAR from *Helicoverpa assulta* wherein the signal peptide has been modified to HDEL) or a variant thereof having at least 75% homology thereto, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such

as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%,
such as at least 89%, such as at least 90%, such as at least 91%, such as at least
92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at
least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as
5 100% homology to the modified Has_FAR as set forth in SEQ ID NO: 31.

In another embodiment, the FAR is Hs_FAR (SEQ ID NO: 33, FAR from *Heliothis
subflexa*) or a variant thereof having at least 75% homology to Hs_FAR, such as at
least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as
10 at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such
as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%,
such as at least 88%, such as at least 89%, such as at least 90%, such as at least
91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at
least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as
15 at least 99%, such as 100% homology to Hs_FAR (SEQ ID NO: 33).

In another embodiment, the FAR is a modified Hs_FAR (SEQ ID NO: 35, FAR from
Heliothis subflexa wherein the signal peptide has been modified to HDEL) or a variant
thereof having at least 75% homology thereto, such as at least 76%, such as at least
20 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at
least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as
at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such
as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%,
such as at least 93%, such as at least 94%, such as at least 95%, such as at least
25 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100%
homology to the modified Hs_FAR as set forth in SEQ ID NO: 35.

In another embodiment, the FAR is Ban_FAR (SEQ ID NO: 45, FAR from *Bicyclus
anyana*) or a variant thereof having at least 75% homology to Hs_FAR, such as at
least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as
30 at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such
as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%,
such as at least 88%, such as at least 89%, such as at least 90%, such as at least
91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at
least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as
35 at least 99%, such as 100% homology to Ban_FAR (SEQ ID NO: 45).

In one embodiment, the FAR is selected from a FAR having at least 60% homology to SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35 or SEQ ID NO: 45. In another embodiment, the FAR is selected from a FAR having at least 60% homology to SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33 or SEQ ID NO: 35. In another embodiment, the FAR is selected from a FAR having at least 60% homology to SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35 or SEQ ID NO: 45. In another embodiment, the FAR is selected from a FAR having at least 60% homology to SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 33, SEQ ID NO: 35 or SEQ ID NO: 45. In another embodiment, the FAR is selected from a FAR having at least 60% homology to SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31 or SEQ ID NO: 45.

In some embodiments, expression of the desaturase and/or of the FAR can be induced, for example if the genes encoding these enzymes are under the control of inducible promoters, as is known in the art. The yeast cell is incubated under suitable conditions, such as in an appropriate medium and at an appropriate temperature as is known to a person of skill in the art. Suitable media supporting yeast growth are known in the art and include, but are not limited to: undefined, complete media such as YEPD (or YPD, Yeast Extract Peptone Dextrose); defined, complete medium such as SC (Synthetic Complete); defined, drop-out medium such as SD (Synthetic Dextrose) lacking one or more elements such as an amino acid or an inducer; or mineral medium, consisting of salts, vitamins and a carbon source, and others.

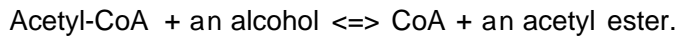
A heterologous fatty acyl-CoA reductase may be expressed from a nucleic acid introduced in the cell, e.g. on a vector such as a plasmid, or by genomic integration. The nucleic acid may be codon-optimised as is known in the art for the specific yeast cell used.

In some embodiments, the yeast cell may express at least two, such as two, heterologous reductases. In a specific embodiment, the yeast cell expresses the reductase from *H. armigera* and the reductase from *H. subflexa*, or variants thereof as described herein.

Acetyltransferase (EC 2.3. 1.84)

The term "acetyltransferase" refers to enzymes of EC number 2.3.1 .84 and can also be termed "alcohol-O-acetyltransferase" or "AcT". The enzyme acts on aliphatic alcohols, and catalyses the reaction (figure 1):

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The yeast cell of the present disclosure preferably overexpresses an acetyltransferase. The acetyltransferase may be a native acetyltransferase which the cell to be modified is already capable of expressing, or it may be a heterologous acetyltransferase. If the yeast cell expresses a native acetyltransferase, the yeast cell is preferably modified so that expression of the native acetyltransferase is increased. This can be done by methods known in the art, such as but not limited to introduction of additional copies of the nucleic acid encoding the acetyltransferase in the genome or on a vector, modification of the promoter to a constitutive promoter with a high expression level, or to an inducible promoter which upon induction leads to high expression levels.

If the yeast cell does not express a native acetyltransferase or if the activity of the native acetyltransferase is insufficient, resulting in low titres, a nucleic acid encoding a heterologous acetyltransferase may be introduced in the cell, either in a genomic location or on a vector, to enable expression of the acetyltransferase. Preferably, the acetyltransferase is expressed at a high level, e.g. by introducing multiple copies of the nucleic acid encoding the acetyltransferase, or by taking advantage of a constitutive promoter with a high expression level, or of an inducible promoter which upon induction leads to high expression levels. The acetyltransferase may be expressed from a nucleic acid introduced in the cell, e.g. on a vector such as a plasmid, or by genomic integration. The nucleic acid may be codon-optimised as is known in the art for the specific yeast cell used.

The term "overexpress" thus refers to the overexpression of an acetyltransferase in a yeast cell when compared to a yeast cell which has not been modified to overexpress the acetyltransferase, i.e. the parent strain.

In some embodiments, the acetyltransferase is the AcT of SEQ ID NO: 21 (Atf1, the *S. cerevisiae* AcT) or a variant thereof having at least 75% homology to Sc_At1, such as

at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to SEQ ID NO: 21.

In other embodiments, the conversion of at least part of the desaturated fatty alcohols produced by the present yeast cells to desaturated fatty acyl acetates is done chemically, as is known to the skilled person. For example, acetyl chloride can be added to the fatty alcohol and the mixture incubated at room temperature after mixing.

Production of a desaturated fatty alcohol

The yeast cells of the present disclosure can be used for the production of a desaturated fatty alcohol and optionally a desaturated fatty acyl acetate. The yeast cell preferably expresses:

- i) at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA having a carbon chain length of 14; and
- ii) at least one heterologous fatty acyl-CoA reductase (FAR), capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol; and
- iii) optionally an acetyltransferase capable of converting at least part of said desaturated fatty alcohol to a desaturated fatty acyl acetate;

wherein the desaturase has a higher specificity towards tetradecanoyl-CoA than towards hexadecanoyl-CoA and/or wherein the fatty acyl-CoA reductase has a higher specificity towards desaturated tetradecanoyl-CoA than towards desaturated hexadecanoyl-CoA.

The yeast cell, the desaturase, the fatty acyl-CoA reductase and the acetyltransferase may all be as described above.

The yeast cell of the present disclosure may thus be used for the production of a range of desaturated fatty alcohols, such as:

- (Z)- Δ^5 desaturated fatty alcohols having a carbon chain length of 14;

- (E)- Δ 5 desaturated fatty alcohols having a carbon chain length of 14;
- (Z)- Δ 6 desaturated fatty alcohols having a carbon chain length of 14;
- (E)- Δ 6 desaturated fatty alcohols having a carbon chain length of 14;
- (Z)- Δ 7 desaturated fatty alcohols having a carbon chain length of 14;
- 5 - (E)- Δ 7 desaturated fatty alcohols having a carbon chain length of 14;
- (Z)- Δ 8 desaturated fatty alcohols having a carbon chain length of 14;
- (E)- Δ 8 desaturated fatty alcohols having a carbon chain length of 14;
- (Z)- Δ 9 desaturated fatty alcohols having a carbon chain length of 14;
- (E)- Δ 9 desaturated fatty alcohols having a carbon chain length of 14;
- 10 - (Z)- Δ 10 desaturated fatty alcohols having a carbon chain length of 14;
- (E)- Δ 10 desaturated fatty alcohols having a carbon chain length of 14;
- (Z)- Δ 11 desaturated fatty alcohols having a carbon chain length of 14;
- (E)- Δ 11 desaturated fatty alcohols having a carbon chain length of 14;
- (Z)- Δ 12 desaturated fatty alcohols having a carbon chain length of 14;
- 15 - (E)- Δ 12 desaturated fatty alcohols having a carbon chain length of 14;
- (Z)- Δ 13 desaturated fatty alcohols having a carbon chain length of 14; and
- (E)- Δ 13 desaturated fatty alcohols having a carbon chain length of 14.

20 The yeast cell disclosed herein may thus express a heterologous Δ 9 desaturase and a fatty acyl-CoA reductase, and be used to produce (Z)9-C14:OH, i.e. a fatty alcohol having a carbon chain length of 14 harbouring a desaturation in Zconformation at position 9. This fatty alcohol is a precursor of (Z)9-C14:OAc, which is an important component of pheromones derived from various species, for example the fall armyworm *Spodoptera frugiperda*.

25 In other embodiments, the yeast cell expresses a heterologous Δ 11 desaturase and a fatty acyl-CoA reductase, and can be used to produce (Z)11-C14:OH, i.e. a fatty alcohol having a carbon chain length of 14 harbouring a desaturation in Zconformation at position 11. This fatty alcohol is a precursor of (Z)11-C14:OAc, which is an important

30 component of pheromones derived from various species, for example the European corn borer *Ostrinia nubilalis* and the red-banded leafroller *Argyrotaenia velutinana*.

35 In other embodiments, the yeast cell expresses a heterologous Δ 11 desaturase and a fatty acyl-CoA reductase, and can be used to produce (E)11-C14:OH, i.e. a fatty alcohol having a carbon chain length of 14 harbouring a desaturation in E conformation at position 11. This fatty alcohol is a precursor of (E)11-C14:OAc, which is an important

component of pheromones derived from various species, for example the lightbrown apple moth *Epiphyas postvittana*.

5 The desaturated fatty alcohols produced by the present yeast cell may be desaturated in more than one position. The desaturated fatty alcohols may be desaturated in at least two positions, such as at least three positions, such as four positions.

10 For example, (E)7, (Z)9 desaturated fatty alcohols may be produced having a carbon chain length of 14. (E)3, (Z)8, (2)1 1 desaturated fatty alcohols may be produced having a carbon chain length of 14. (Z)9, (E)1 1, (E)13 desaturated fatty alcohols may be produced having a carbon chain length of 14.

15 The thus produced desaturated fatty alcohols may be further modified as is known in the art, for example by carbon chain shortening, in order to obtain desaturated fatty alcohols having a carbon chain of less than 14, such as 12, 10, 8, 6 or 4. Thus, (E)7, (Z)9 desaturated fatty alcohols may be produced having a carbon chain length of 12, (E)3, (2)8, (Z)1 1 desaturated fatty alcohols may be produced having a carbon chain length of 12, and (Z)9, (E)1 1, (E)13 desaturated fatty alcohols may be produced having a carbon chain length of 12.

20 In order to further increase production of desaturated fatty alcohols, it may be beneficial to mutate one or more genes encoding a lipase so that the corresponding lipase has partial or total loss of activity. Accordingly, in some embodiments, the yeast cell may be as described herein and additionally carry one or more mutations resulting in total or partial loss of activity of one or more lipases.

25 It is known in the art that there are numerous genes encoding lipases. Their expression and/or activity may be a function of the medium in which the yeast cell is cultivated. Accordingly, the choice of medium may help choosing which lipase gene should be deleted or mutated in order for the corresponding lipase to have reduced or total loss of activity in said medium.

30 Several lipases may be active in one medium at the same time. Thus, in some embodiments, the yeast cell has several mutations, resulting in total or partial loss of activity of several lipases. In order to limit degradation of fatty acyl acetate, in some

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embodiments the yeast cell has several mutations resulting in total or partial loss of activity of all the lipases known to be or suspected of being active in a given medium.

By way of example, lipase 2, lipase 5 and lipase 8 are the major lipases active in
5 *Yarrowia lipolytica* when the cells are grown on glucose. Accordingly, if a glucose-based medium is employed, total or partial loss of activity of one, two or all of lipase 2, lipase 5 and lipase 8 may be considered.

In some embodiments, the lipase has at least 60% homology to lipase 2 of *Y. lipolytica*
10 as set forth in SEQ ID NO: 72, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%,
15 such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%,
20 such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology. In other embodiments, the lipase has at least 60% homology to lipase 7 of *Y. lipolytica* as set forth in SEQ ID NO: 73, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology,
25 such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%,
30 such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology. In other embodiments, the lipase has at least 60% homology to lipase 8 of *Y. lipolytica* as set forth in SEQ ID NO: 74, such as at least 61% homology,

such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%,
5 such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%,
10 such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology.

In some embodiments, the yeast cell has:

- 15
- a mutation resulting in total or partial loss of activity of a lipase having at least 60% homology to lipase 2 of *Y. lipolytica* as set forth in SEQ ID NO: 72; and
 - a mutation resulting in total or partial loss of activity of a lipase having at least 60% homology to lipase 7 of *Y. lipolytica* as set forth in SEQ ID NO: 73.

20 In other embodiments, the yeast cell has:

- a mutation resulting in total or partial loss of activity of a lipase having at least 60% homology to lipase 2 of *Y. lipolytica* as set forth in SEQ ID NO: 72; and
- a mutation resulting in total or partial loss of activity of a lipase having at least 60% homology to lipase 8 of *Y. lipolytica* as set forth in SEQ ID NO: 74.

25

In other embodiments, the yeast cell has:

- a mutation resulting in total or partial loss of activity of a lipase having at least 60% homology to lipase 7 of *Y. lipolytica* as set forth in SEQ ID NO: 73; and
- a mutation resulting in total or partial loss of activity of a lipase having at least
30 60% homology to lipase 8 of *Y. lipolytica* as set forth in SEQ ID NO: 74.

In some embodiments, the yeast cell has:

- a mutation resulting in total or partial loss of activity of a lipase having at least 60% homology to lipase 2 of *Y. lipolytica* as set forth in SEQ ID NO: 72; and
- 35 - a mutation resulting in total or partial loss of activity of a lipase having at least 60% homology to lipase 7 of *Y. lipolytica* as set forth in SEQ ID NO: 73; and

- a mutation resulting in total or partial loss of activity of a lipase having at least 60% homology to lipase 8 of *Y. lipolytica* as set forth in SEQ ID NO: 74.

Production of a desaturated fatty acyl acetate

- 5 The yeast cell of the present disclosure may optionally express or overexpress a native or a heterologous acetyltransferase capable of converting at least part of the desaturated fatty alcohols produced by the cell in desaturated fatty acyl acetates, and may thus be used for the production of a range of desaturated fatty acetates, such as:
- (Z)- Δ 5 desaturated fatty acetates having a carbon chain length of 14;
 - 10 - (E)- Δ 5 desaturated fatty acetates having a carbon chain length of 14;
 - (Z)- Δ 6 desaturated fatty acetates having a carbon chain length of 14;
 - (E)- Δ 6 desaturated fatty acetates having a carbon chain length of 14;
 - (Z)- Δ 7 desaturated fatty acetates having a carbon chain length of 14;
 - (E)- Δ 7 desaturated fatty acetates having a carbon chain length of 14;
 - 15 - (Z)- Δ 8 desaturated fatty acetates having a carbon chain length of 14;
 - (E)- Δ 8 desaturated fatty acetates having a carbon chain length of 14;
 - (Z)- Δ 9 desaturated fatty acetates having a carbon chain length of 14;
 - (E)- Δ 9 desaturated fatty acetates having a carbon chain length of 14;
 - (Z)- Δ 10 desaturated fatty acetates having a carbon chain length of 14;
 - 20 - (E)- Δ 10 desaturated fatty acetates having a carbon chain length of 14;
 - (Z)- Δ 11 desaturated fatty acetates having a carbon chain length of 14;
 - (E)- Δ 11 desaturated fatty acetates having a carbon chain length of 14;
 - (Z)- Δ 12 desaturated fatty acetates having a carbon chain length of 14;
 - (E)- Δ 12 desaturated fatty acetates having a carbon chain length of 14;
 - 25 - (Z)- Δ 13 desaturated fatty acetates having a carbon chain length of 14; and
 - (E)- Δ 13 desaturated fatty acetates having a carbon chain length of 14.

Accordingly, in one embodiment, the yeast cell expresses a heterologous Δ 9 desaturase, a heterologous FAR and an acetyltransferase and can be used to obtain

30 (Z)9-C14:OAc, i.e. a fatty acyl acetate having a carbon chain length of 14 harbouring a desaturation in Zconformation at position 9. This fatty acyl acetate is an important component of pheromones derived from various species, for example the fall armyworm *Spodoptera frugiperda*.

In other embodiments, the yeast cell expresses a heterologous $\Delta 11$ desaturase, a heterologous FAR and an acetyltransferase, and can be used to produce (Z)¹¹ 1-C₁₄:OAc, i.e. a fatty acyl acetate having a carbon chain length of 14 harbouring a desaturation in Z conformation at position 11. This fatty acyl acetate is an important component of pheromones derived from various species, for example the European corn borer *Ostrinia nubilalis* and the red-banded leafroller *Argyrotaenia velutinana*.

In other embodiments, the yeast cell expresses a heterologous $\Delta 11$ desaturase, a heterologous FAR and an acetyltransferase, and can be used to produce (E)¹¹ 1-C₁₄:OAc, i.e. a fatty acyl acetate having a carbon chain length of 14 harbouring a desaturation in E conformation at position 11. This fatty acyl acetate is an important component of pheromones derived from various species, for example the lightbrown apple moth *Epiphyas postvittana*.

The desaturated fatty acetates produced by the present yeast cell may be desaturated in more than one position. The desaturated fatty acetates may be desaturated in at least two positions, such as at least three positions, such as four positions.

For example, (E)⁷, (Z)⁹ desaturated fatty acetates may be produced having a carbon chain length of 14. (E)³, (Z)⁸, (Z)¹¹ 1 desaturated fatty acetates may be produced having a carbon chain length of 14. (Z)⁹, (E)¹¹ 1, (E)¹³ desaturated fatty acetates may be produced having a carbon chain length of 14.

The thus produced desaturated fatty acetates may be further modified as is known in the art, for example by carbon chain shortening, in order to obtain desaturated fatty acetates having a carbon chain of less than 14, such as 12, 10, 8, 6 or 4. Thus, (E)⁷, (Z)⁹ desaturated fatty acetates may be produced having a carbon chain length of 12, (E)³, (Z)⁸, (Z)¹¹ 1 desaturated fatty acetates may be produced having a carbon chain length of 12, and (Z)⁹, (E)¹¹ 1, (E)¹³ desaturated fatty acetates may be produced having a carbon chain length of 12.

Production of a desaturated fatty aldehyde

While the present disclosure provides methods for producing desaturated fatty alcohols and desaturated fatty acyl acetates, it may be of interest to further convert said fatty alcohols to the corresponding aldehydes. Thus in some embodiments, the method may

further comprise the step of converting at least part of the fatty alcohols to fatty aldehydes, thereby producing fatty aldehydes. This can be achieved by chemical methods or by further engineering of the yeast cell.

5 In some embodiments, the step of converting at least part of the fatty alcohols to the corresponding aldehydes is a step of chemical conversion. The chemical conversion is based on the oxidation of fatty alcohols to the corresponding aldehydes. Methods for performing this conversion are known in the art. Preferred methods are environmentally friendly and minimize the amount of hazardous waste.

10

Thus in some embodiments, the chemical conversion may be metal free, avoiding toxic heavy metal based reagents such as manganese oxides, chromium oxides (Jones ox. PDC, PCC) or ruthenium compounds (TPAP, Ley-Griffith ox.). In some embodiments, the conversion does not involve reactions with activated dimethyl sulfoxide such as the Swern oxidation or the Pfitzner-Moffat type. Such reactions may involve the stereotypic formation of traces of intensively smelling organic sulfur compounds such as dimethyl sulfide which can be difficult to remove from the target product.

15

In some embodiments, the method comprises a Dess-Martin reaction (Yadav et al., 2004, Meyer et al., 1994). In some embodiments, the method comprises a Copper(I)/ABNO-catalysed aerobic alcohol oxidation reaction (Steves & Stahl, 2013).

20

In other embodiments, the chemical conversion comprises the oxidation with sodium hypochlorite under aqueous/organic two phase conditions (Okada et al., 2014; Tamura et al., 2012; Li et al., 2009). In some embodiments, the chemical oxidation can be performed with 1-chlorobenzotriazole in a medium of methylene chloride containing 25% pyridine (Ferrell and Yao, 1972).

25

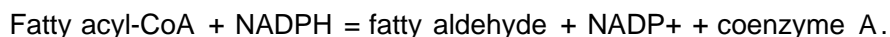
Alternatively, the oxidation of a fatty alcohol to the corresponding fatty aldehyde can be performed enzymatically by alcohol dehydrogenases. The skilled person will know how to carry out enzymatic oxidation. For example, enzymatic oxidation can be carried out by contacting purified enzymes, cell extracts or whole cells, with the fatty alcohol.

30

The fatty alcohols obtainable by the cells and methods described herein can be further converted in fatty aldehydes by introducing a gene encoding an aldehyde-forming fatty acyl-CoA reductase EC 1.2.1 .50 (FAR'). In this way, at least part of the desaturated

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fatty acyl-CoA can be converted to the corresponding fatty aldehyde by an aldehyde-forming fatty acyl-CoA reductase (FAR'). The enzymes capable of catalyzing this conversion can catalyse a reduction reaction, where the fatty acyl-CoA is reduced to a fatty aldehyde. Such enzymes are aldehyde-forming fatty acyl-CoA reductases, herein
 5 also referred to as FAR' or "aldehyde-forming FAR' ", with an EC number 1.2.1 .50. They catalyse the following reaction:



10 In some embodiments, expression of the aldehyde-forming FAR' can be induced, for example if the gene encoding this enzyme is under the control of inducible promoters, as is known in the art. The yeast cell is incubated under suitable conditions, such as in an appropriate medium and at an appropriate temperature as is known to a person of skill in the art. Suitable media supporting yeast growth are known in the art and include,
 15 but are not limited to: undefined, complete media such as YEPD (or YPD, Yeast Extract Peptone Dextrose), defined, complete medium such as SC (Synthetic Complete), or defined, drop-out medium such as SD (Synthetic Dextrose) lacking one or more elements such as an amino acid or an inducer.

20 Thus, the following aldehydes can be obtained:

- (Z)- Δ^5 desaturated fatty aldehydes having a carbon chain length of 14;
- (E)- Δ^5 desaturated fatty aldehydes having a carbon chain length of 14;
- (Z)- Δ^6 desaturated fatty aldehydes having a carbon chain length of 14;
- (E)- Δ^6 desaturated fatty aldehydes having a carbon chain length of 14;
- 25 - (Z)- Δ^7 desaturated fatty aldehydes having a carbon chain length of 14;
- (E)- Δ^7 desaturated fatty aldehydes having a carbon chain length of 14;
- (Z)- Δ^8 desaturated fatty aldehydes having a carbon chain length of 14;
- (E)- Δ^8 desaturated fatty aldehydes having a carbon chain length of 14;
- (Z)- Δ^9 desaturated fatty aldehydes having a carbon chain length of 14;
- 30 - (E)- Δ^9 desaturated fatty aldehydes having a carbon chain length of 14;
- (Z)- Δ^{10} desaturated fatty aldehydes having a carbon chain length of 14;
- (E)- Δ^{10} desaturated fatty aldehydes having a carbon chain length of 14;
- (Z)- Δ^{11} desaturated fatty aldehydes having a carbon chain length of 14;
- (E)- Δ^{11} desaturated fatty aldehydes having a carbon chain length of 14;
- 35 - (Z)- Δ^{12} desaturated fatty aldehydes having a carbon chain length of 14;
- (E)- Δ^{12} desaturated fatty aldehydes having a carbon chain length of 14;

- (Z)- Δ 13 desaturated fatty aldehydes having a carbon chain length of 14; and
- (E)- Δ 13 desaturated fatty aldehydes having a carbon chain length of 14.

5 The desaturated fatty aldehydes produced by the present yeast cell may be desaturated in more than one position. The desaturated fatty aldehydes may be desaturated in at least two positions, such as at least three positions, such as four positions.

10 For example, (E)7, (Z)9 desaturated fatty aldehydes may be produced having a carbon chain length of 14. (E)3, (Z)8, (Z)11 desaturated fatty aldehydes may be produced having a carbon chain length of 14. (Z)9, (E)11, (E)13 desaturated fatty aldehydes may be produced having a carbon chain length of 14.

15 The thus produced desaturated fatty aldehydes may be further modified as is known in the art, for example by carbon chain shortening, in order to obtain desaturated fatty aldehydes having a carbon chain of less than 14, such as 12, 10, 8, 6 or 4. Thus, (E)7, (Z)9 desaturated fatty aldehydes may be produced having a carbon chain length of 12, (E)3, (Z)8, (Z)11 desaturated fatty aldehydes may be produced having a carbon chain length of 12, and (Z)9, (E)11, (E)13 desaturated fatty aldehydes may be produced
20 having a carbon chain length of 12.

Fatty acyl-CoA

In order for the yeast cell to produce desaturated fatty alcohols and desaturated fatty acyl acetates as described herein, the yeast cell needs to be provided with fatty acyl-CoAs as a substrate. Preferably, the fatty acyl-CoA has a carbon chain length of 14
25 and is myristoyl-CoA.

30 Such fatty acyl-CoA can either be provided in the medium in which the yeast cell is incubated, or the yeast cell may be naturally able to produce such fatty acyl-CoA, or the yeast cell may be engineered in order to produce or to increase production of such fatty acyl-CoA. Preferably, the yeast cell is provided with or is capable of producing myristoyl-CoA.

35 In some embodiments, the yeast cell is not naturally capable of producing a fatty acyl-CoA having a carbon chain length of 14. The yeast cell may in this case be engineered

as is known in the art, for example by the introduction of a heterologous thioesterase. Thus in some embodiments, a nucleic acid encoding a thioesterase is introduced in the yeast cell, on a vector or by genomic integration. The thioesterase gene may be under the control of an inducible promoter, or under the control of a constitutive promoter.

5 The nucleic acid encoding a thioesterase may be codon-optimised for the yeast cell, as is known in the art. In particular, the nucleic acid may be codon-optimised for a *Yarrowia* cell, such as a *Yarrowia lipolytica* cell.

In some embodiments, the thioesterase is derived from an organism selected from
10 *Cuphea palustris*, *Cuphea hookeriana*, *Cinnamomum camphora*, or from *Escherichia coli*. In preferred embodiments, the thioesterase is derived from *Escherichia coli* or *Cinnamomum camphora*. In some embodiments, the thioesterase has at least 60% homology to a thioesterase selected from the thioesterase derived from *Cuphea*
15 *palustris* as set forth in SEQ ID NO: 23, the thioesterase derived from *Cuphea hookeriana* as set forth in SEQ ID NO: 38, the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40, and the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42. Preferably, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40 or from *Escherichia coli* as set forth in SEQ ID NO: 42. In one
20 embodiment, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40. In another embodiment the thioesterase has at least 60% homology to the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42.

25 In another embodiment, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology,
30 such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least
35 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as

at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40.

5 In another embodiment, the thioesterase has at least 60% homology to the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at
10 least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such
15 as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42.

20

The nucleic acid encoding a thioesterase may be codon-optimised as is known in the art. In one embodiment, the yeast cell is a *Yarrowia* cell, preferably a *Yarrowia lipolytica* cell, and the nucleic acid is codon-optimised accordingly.

25 In one embodiment, the at least one thioesterase is encoded by a nucleic acid having at least 60% homology to the nucleic acid encoding the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 39, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology,
30 such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%,
35 such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at

least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to the nucleic acid encoding the thioesterase from *Cinnamomum camphora* as set forth in SEQ ID NO: 39.

5

In one embodiment, the at least one thioesterase is encoded by a nucleic acid having at least 60% homology to the nucleic acid encoding the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 41, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to the nucleic acid encoding the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 41.

In some embodiments, availability of fatty acids having a chain length of 14 may be increased or further increased. For instance, the fatty acid synthase complex may be engineered so that formation of C14-fatty acyl-CoA is increased. The fatty acid synthase complex (EC 2.3.1.86) consists of two subunits, Fas1 (beta subunit) and Fas2 (alpha subunit). The alpha subunit comprises a ketoacyl synthase domain (a "binding pocket") which is hypothesized to be involved in determining the length of the synthesized fatty acids. In *Yarrowia lipolytica*, the native (wild-type) FAS2 is as set forth in SEQ ID NO: 71.

Accordingly, in order to direct the metabolic flux towards production of desaturated fatty alcohols, acetates or aldehydes having a chain length of 14 C, the yeast cell may further express a fatty acyl synthase variant having a modified ketone synthase domain. Without being bound by theory, it is hypothesized that the modified ketone synthase domain results in a modified binding pocket, which thus more readily

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accommodates medium length substrates such as C14 substrates, thereby producing a higher proportion of C 14 products.

5 In one embodiment, the yeast cell is a *Yarrowia lipolytica* cell as described herein, wherein the cell further expresses a modified fatty acid synthase complex. In one embodiment, the fatty acid synthase complex is modified by mutating the gene encoding the alpha subunit of the complex. In some embodiments, the mutation is in the gene encoding FAS2. The mutation may result in modification of one or more of residue 1220 (I1220), residue 1217 (M1217) or residue 1226 (M1 226) of SEQ ID NO:
10 71, resulting in a variant FAS2. The skilled person will know how to design such mutations.

Preferably, the mutation results in an I1220F variant, an I1220W variant, an I1220Y variant or an I1220H variant. In a specific embodiment, the mutation results in an
15 I1220F variant. In some embodiments, the mutation results in an M1217F variant, an M1217W variant, an M1217Y variant or an M1217H variant. In other embodiments, the mutation results in an M1226F variant, an M1226W variant, an M1226Y variant or an M1226H variant. Yeast cells with more than one of the above mutations are also contemplated, such as two mutations or three mutations at residue I1220, M1217 or
20 M1226.

Yeast cell

The present disclosure provides a yeast cell which has been modified to produce a desaturated fatty alcohol, and optionally a desaturated fatty acyl acetate. Desaturated fatty alcohols and desaturated fatty acyl acetates are components of pheromones, in
25 particular of moth pheromones. The yeast cell disclosed herein thus provides a platform for environment-friendly moth pheromone production.

The yeast cell may be a non-naturally occurring yeast cell, for example a yeast cell which has been engineered to produce desaturated fatty alcohols and desaturated fatty
30 acyl acetates.

In some embodiments, the cell has been modified at the genomic level, e.g. by gene editing in the genome. The cell may also be modified by insertion of at least one nucleic acid construct such as at least one vector. The vector may be designed as is
35 known to the skilled person to either enable integration of nucleic acid sequences in the

genome, or to enable expression of a polypeptide encoded by a nucleic acid sequence comprised in the vector without genome integration.

5 The yeast cell may be of a genus selected from *Saccharomyces*, *Pichia*, *Yarrowia*, *Kluyveromyces*, *Candida*, *Rhodotorula*, *Rhodospiridium*, *Cryptococcus*, *Trichosporon* and *Lipomyces*. In a preferred embodiment, the genus is *Saccharomyces* or *Yarrowia*, most preferably the genus is *Yarrowia*.

10 The yeast cell may be of a species selected from *Saccharomyces cerevisiae*, *Pichia pastoris*, *Kluyveromyces marxianus*, *Cryptococcus albidus*, *Lipomyces lipofera*, *Lipomyces starkeyi*, *Rhodospiridium toruloides*, *Rhodotorula glutinis*, *Trichosporon pullulan* and *Yarrowia lipolytica*. In preferred embodiments, the yeast cell is a *Saccharomyces cerevisiae* cell or a *Yarrowia lipolytica* cell, most preferably the yeast cell is a *Yarrowia lipolytica* cell.

15 The yeast cell to be modified, which will also be referred to as the host cell, may express native enzymes which are of the same class than the enzymes which are necessary for the production of desaturated fatty alcohols and desaturated fatty acyl acetates. In some cases, however, such native enzymes may have a negative impact on the titre of desaturated fatty alcohols and/or desaturated fatty acyl acetates which can be obtained; the native enzymes may thus be inactivated by methods known in the art, such as gene editing. For example, the genes encoding the native enzymes having a negative impact on the titre may be deleted or mutated so as to lead to total or partial loss of activity of the native enzyme.

25 The yeast cell of the present disclosure express at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA having a carbon chain length of 14 as described herein, at least one heterologous fatty acyl-CoA reductase capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol as described herein, and optionally an acetyltransferase capable of converting at least part of said desaturated fatty alcohol to a desaturated fatty acyl acetate, wherein the desaturase has a higher specificity towards tetradecanoyl-CoA than towards hexadecanoyl-CoA and/or wherein the fatty acyl-CoA reductase has a higher specificity towards desaturated tetradecanoyl-CoA than
30 towards desaturated hexadecanoyl-CoA. In some embodiments, the yeast also

expresses an acetyltransferase. In some embodiments, the yeast also expresses a thioesterase.

In one embodiment, the yeast cell expresses:

- 5 i) at least one heterologous $\Delta 3$ desaturase; and
- ii) at least one heterologous FAR; and
- iii) optionally overexpresses an acetyltransferase, and
- iv) optionally overexpresses a thioesterase,

wherein the $\Delta 3$ desaturase has a higher specificity towards tetradecanoyl-CoA than
 10 towards hexadecanoyl-CoA and/or wherein the fatty acyl-CoA reductase has a higher
 specificity towards desaturated tetradecanoyl-CoA than towards desaturated
 hexadecanoyl-CoA, whereby the yeast cell produces a fatty alcohol having a carbon
 chain length of 14 and desaturated in position 3. The acetyltransferase may be the AcT
 of SEQ ID NO: 21 (Atf1, the *S. cerevisiae* AcT) or a variant thereof having at least 75%
 15 homology to Sc_At1, such as at least 76%, such as at least 77%, such as at least
 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at
 least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as
 at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such
 as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%,
 20 such as at least 94%, such as at least 95%, such as at least 96%, such as at least
 97%, such as at least 98%, such as at least 99%, such as 100% homology to SEQ ID
 NO: 21. In some embodiments, the thioesterase has at least 60% homology to a
 thioesterase selected from the thioesterase derived from *Cuphea palustris* as set forth
 in SEQ ID NO: 23, the thioesterase from *Cuphea hookeriana* as set forth in SEQ ID
 25 NO: 38, the thioesterase from *Cinnamomum camphora* as set forth in SEQ ID NO: 40,
 and the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 42. Preferably,
 the thioesterase is derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40
 or from *Escherichia coli* as set forth in SEQ ID NO: 42. In one embodiment, the
 thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum*
 30 *camphora* as set forth in SEQ ID NO: 40. In another embodiment the thioesterase has
 at least 60% homology to the thioesterase derived from *Escherichia coli* as set forth in
 SEQ ID NO: 42.

In one embodiment, the yeast cell expresses:

- 35 i) at least one heterologous $\Delta 5$ desaturase; and
- ii) at least one heterologous FAR; and

- iii) optionally overexpresses an acetyltransferase, and
- iv) optionally overexpresses a thioesterase,

wherein the $\Delta 5$ desaturase has a higher specificity towards tetradecanoyl-CoA than towards hexadecanoyl-CoA and/or wherein the fatty acyl-CoA reductase has a higher specificity towards desaturated tetradecanoyl-CoA than towards desaturated hexadecanoyl-CoA, whereby the yeast cell produces a fatty alcohol having a carbon chain length of 14 and desaturated in position 5. The acetyltransferase may be the AcT of SEQ ID NO: 21 (Atf1, the *S. cerevisiae* AcT) or a variant thereof having at least 75% homology to Sc_At1, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to SEQ ID NO: 21. In some embodiments, the thioesterase has at least 60% homology to a thioesterase selected from the thioesterase derived from *Cuphea palustris* as set forth in SEQ ID NO: 23, the thioesterase from *Cuphea hookeriana* as set forth in SEQ ID NO: 38, the thioesterase from *Cinnamomum camphora* as set forth in SEQ ID NO: 40, and the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 42. Preferably, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40 or from *Escherichia coli* as set forth in SEQ ID NO: 42. In one embodiment, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40. In another embodiment the thioesterase has at least 60% homology to the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42.

In one embodiment, the yeast cell expresses:

- i) at least one heterologous $\Delta 6$ desaturase; and
- ii) at least one heterologous FAR; and
- iii) optionally overexpresses an acetyltransferase, and
- iv) optionally overexpresses a thioesterase,

wherein the $\Delta 6$ desaturase has a higher specificity towards tetradecanoyl-CoA than towards hexadecanoyl-CoA and/or wherein the fatty acyl-CoA reductase has a higher specificity towards desaturated tetradecanoyl-CoA than towards desaturated hexadecanoyl-CoA, whereby the yeast cell produces a fatty alcohol having a carbon

chain length of 14 and desaturated in position 6. The acetyltransferase may be the AcT of SEQ ID NO: 21 (Atf1, the *S. cerevisiae* AcT) or a variant thereof having at least 75% homology to Sc_At1, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to SEQ ID NO: 21. In some embodiments, the thioesterase has at least 60% homology to a thioesterase selected from the thioesterase derived from *Cuphea palustris* as set forth in SEQ ID NO: 23, the thioesterase from *Cuphea hookeriana* as set forth in SEQ ID NO: 38, the thioesterase from *Cinnamomum camphora* as set forth in SEQ ID NO: 40, and the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 42. Preferably, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40 or from *Escherichia coli* as set forth in SEQ ID NO: 42. In one embodiment, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40. In another embodiment the thioesterase has at least 60% homology to the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42.

In one embodiment, the yeast cell expresses:

- i) at least one heterologous $\Delta 7$ desaturase; and
- ii) at least one heterologous FAR; and
- iii) optionally overexpresses an acetyltransferase, and
- iv) optionally overexpresses a thioesterase,

wherein the $\Delta 7$ desaturase has a higher specificity towards tetradecanoyl-CoA than towards hexadecanoyl-CoA and/or wherein the fatty acyl-CoA reductase has a higher specificity towards desaturated tetradecanoyl-CoA than towards desaturated hexadecanoyl-CoA, whereby the yeast cell produces a fatty alcohol having a carbon chain length of 14 and desaturated in position 7. The acetyltransferase may be the AcT of SEQ ID NO: 21 (Atf1, the *S. cerevisiae* AcT) or a variant thereof having at least 75% homology to Sc_At1, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such

as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%,
 such as at least 94%, such as at least 95%, such as at least 96%, such as at least
 97%, such as at least 98%, such as at least 99%, such as 100% homology to SEQ ID
 NO: 21. In some embodiments, the thioesterase has at least 60% homology to a
 5 thioesterase selected from the thioesterase derived from *Cuphea palustris* as set forth
 in SEQ ID NO: 23, the thioesterase from *Cuphea hookeriana* as set forth in SEQ ID
 NO: 38, the thioesterase from *Cinnamomum camphora* as set forth in SEQ ID NO: 40,
 and the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 42. Preferably,
 the thioesterase has at least 60% homology to the thioesterase derived from
 10 *Cinnamomum camphora* as set forth in SEQ ID NO: 40 or from *Escherichia coli* as set
 forth in SEQ ID NO: 42. In one embodiment, the thioesterase has at least 60%
 homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ
 ID NO: 40. In another embodiment the thioesterase has at least 60% homology to the
 thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42.

15 In one embodiment, the yeast cell expresses:

- i) at least one heterologous $\Delta 8$ desaturase; and
- ii) at least one heterologous FAR; and
- iii) optionally overexpresses an acetyltransferase, and
- 20 iv) optionally overexpresses a thioesterase,

wherein the $\Delta 8$ desaturase has a higher specificity towards tetradecanoyl-CoA than
 towards hexadecanoyl-CoA and/or wherein the fatty acyl-CoA reductase has a higher
 specificity towards desaturated tetradecanoyl-CoA than towards desaturated
 hexadecanoyl-CoA, whereby the yeast cell produces a fatty alcohol having a carbon
 25 chain length of 14 and desaturated in position 8. The acetyltransferase may be the AcT
 of SEQ ID NO: 21 (Atf1, the *S. cerevisiae* AcT) or a variant thereof having at least 75%
 homology to Sc_At1, such as at least 76%, such as at least 77%, such as at least
 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at
 least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as
 30 at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such
 as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%,
 such as at least 94%, such as at least 95%, such as at least 96%, such as at least
 97%, such as at least 98%, such as at least 99%, such as 100% homology to SEQ ID
 NO: 21. In some embodiments, the thioesterase has at least 60% homology to a
 35 thioesterase selected from the thioesterase derived from *Cuphea palustris* as set forth
 in SEQ ID NO: 23, the thioesterase from *Cuphea hookeriana* as set forth in SEQ ID

- NO: 38, the thioesterase from *Cinnamomum camphora* as set forth in SEQ ID NO: 40, and the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 42. Preferably, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40 or from *Escherichia coli* as set forth in SEQ ID NO: 42. In one embodiment, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40. In another embodiment the thioesterase has at least 60% homology to the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42.
- 10 In one embodiment, the yeast cell expresses:
- i) at least one heterologous $\Delta 9$ desaturase; and
 - ii) at least one heterologous FAR; and
 - iii) optionally overexpresses an acetyltransferase, and
 - iv) optionally overexpresses a thioesterase,
- 15 wherein the $\Delta 9$ desaturase has a higher specificity towards tetradecanoyl-CoA than towards hexadecanoyl-CoA and/or wherein the fatty acyl-CoA reductase has a higher specificity towards desaturated tetradecanoyl-CoA than towards desaturated hexadecanoyl-CoA, whereby the yeast cell produces a fatty alcohol having a carbon chain length of 14 and desaturated in position 9. The acetyltransferase may be the Act of SEQ ID NO: 21 (Atf1, the *S. cerevisiae* Act) or a variant thereof having at least 75% homology to Sc_At1, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to SEQ ID NO: 21. In some embodiments, the thioesterase has at least 60% homology to a thioesterase selected from the thioesterase derived from *Cuphea palustris* as set forth in SEQ ID NO: 23, the thioesterase from *Cuphea hookeriana* as set forth in SEQ ID NO: 38, the thioesterase from *Cinnamomum camphora* as set forth in SEQ ID NO: 40, and the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 42. Preferably, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40 or from *Escherichia coli* as set forth in SEQ ID NO: 42. In one embodiment, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ

ID NO: 40. In another embodiment the thioesterase has at least 60% homology to the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42.

In a particular embodiment, the yeast cell expresses:

- 5 - a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 10, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 10; and
- 20 - a FAR having at least 75% homology to Har_FAR as set forth in SEQ ID NO: 25 or SEQ ID NO: 27, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to Har_FAR as set forth in SEQ ID NO: 25 or SEQ ID NO: 27;
- 30 - and optionally expresses or overexpresses an acetyltransferase and/or a thioesterase. Preferably, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40 or from *Escherichia coli* as set forth in SEQ ID NO: 42. In one embodiment,
- 35 the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40. In another embodiment

the thioesterase has at least 60% homology to the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42.

In another particular embodiment, the yeast cell expresses:

- 5 - a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 10, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 10; and
- 20 - a FAR having at least 75% homology to Has_FAR as set forth in SEQ ID NO: 29 or SEQ ID NO: 31, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to Has_FAR as set forth in SEQ ID NO: 29 or SEQ ID NO: 31;
- 30 - and optionally expresses or overexpresses an acetyltransferase and/or a thioesterase. Preferably, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40 or from *Escherichia coli* as set forth in SEQ ID NO: 42. In one embodiment,
- 35 the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40. In another embodiment

the thioesterase has at least 60% homology to the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42.

In another particular embodiment, the yeast cell expresses:

- 5 - a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 10, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68%
10 homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%,
15 such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 10; and
20 - a FAR having at least 75% homology to Hs_FAR as set forth in SEQ ID NO: 33 or SEQ ID NO: 35, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%,
25 such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to Hs_FAR as set forth in SEQ ID NO: 33 or SEQ ID
30 NO: 35;
- and optionally expresses or overexpresses an acetyltransferase and/or a thioesterase. Preferably, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40 or from *Escherichia coli* as set forth in SEQ ID NO: 42. In one embodiment,
35 the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40. In another embodiment

the thioesterase has at least 60% homology to the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42.

In another particular embodiment, the yeast cell expresses:

- 5 - a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 10, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68%
10 homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%,
15 such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 10; and
20 - a FAR having at least 75% homology to Ban_FAR as set forth in SEQ ID NO: 45, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%,
25 such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to Ban_FAR as set forth in SEQ ID NO: 45;
30 - and optionally expresses or overexpresses an acetyltransferase and/or a thioesterase. Preferably, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40 or from *Escherichia coli* as set forth in SEQ ID NO: 42. In one embodiment, the thioesterase has at least 60% homology to the thioesterase derived from
35 *Cinnamomum camphora* as set forth in SEQ ID NO: 40. In another embodiment

the thioesterase has at least 60% homology to the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42.

In a particular embodiment, the yeast cell expresses:

- 5 - a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Spodoptera litura* as set forth in SEQ ID NO: 12, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68%
10 homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at
15 least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to the $\Delta 9$ desaturase from *Spodoptera litura* as set forth in SEQ ID NO: 12; and
20 - a FAR having at least 75% homology to Har_FAR as set forth in SEQ ID NO: 25 or SEQ ID NO: 27, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at
25 least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to Har_FAR as set forth in SEQ ID NO: 25 or SEQ ID NO: 27;
30 - and optionally expresses or overexpresses an acetyltransferase and/or a thioesterase. Preferably, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40 or from *Escherichia coli* as set forth in SEQ ID NO: 42. In one embodiment,
35 the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40. In another embodiment

the thioesterase has at least 60% homology to the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42.

In another particular embodiment, the yeast cell expresses:

- 5 - a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Spodoptera litura* as set forth in SEQ ID NO: 12, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to $\Delta 9$ desaturase from *Spodoptera litura* as set forth in SEQ ID NO: 12; and
- 20 - a FAR having at least 75% homology to Has_FAR as set forth in SEQ ID NO: 29 or SEQ ID NO: 31, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to Has_FAR as set forth in SEQ ID NO: 29 or SEQ ID NO: 31;
- 30 - and optionally expresses or overexpresses an acetyltransferase and/or a thioesterase. Preferably, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40 or from *Escherichia coli* as set forth in SEQ ID NO: 42. In one embodiment,
- 35 the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40. In another embodiment

the thioesterase has at least 60% homology to the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42.

In another particular embodiment, the yeast cell expresses:

- 5 - a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Spodoptera litura* as set forth in SEQ ID NO: 12, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to the $\Delta 9$ desaturase from *Spodoptera litura* as set forth in SEQ ID NO: 12; and
- 20 - a FAR having at least 75% homology to Hs_FAR as set forth in SEQ ID NO: 33 or SEQ ID NO: 35, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to Hs_FAR as set forth in SEQ ID NO: 33 or SEQ ID NO: 35;
- 30 - and optionally expresses or overexpresses an acetyltransferase and/or a thioesterase. Preferably, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40 or from *Escherichia coli* as set forth in SEQ ID NO: 42. In one embodiment,
- 35 the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40. In another embodiment

the thioesterase has at least 60% homology to the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42.

In another particular embodiment, the yeast cell expresses:

- 5 - a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Spodoptera litura* as set forth in SEQ ID NO: 12, such as at least 61% homology, such as at least 62% homology, such as at least 63% homology, such as at least 64% homology, such as at least 65% homology, such as at least 66% homology, such as at least 67% homology, such as at least 68% homology, such as at least 69% homology, such as at least 70% homology, such as at least 71% homology, such as at least 72%, such as at least 73%, such as at least 74%, such as at least 75%, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to the $\Delta 9$ desaturase from *Spodoptera litura* as set forth in SEQ ID NO: 12; and
- 20 - a FAR having at least 75% homology to Ban_FAR as set forth in SEQ ID NO: 45, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to Ban_FAR as set forth in SEQ ID NO: 45;
- 25 - and optionally expresses or overexpresses an acetyltransferase and/or a thioesterase. Preferably, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40 or from *Escherichia coli* as set forth in SEQ ID NO: 42. In one embodiment, the thioesterase has at least 60% homology to the thioesterase derived from
- 30 *Cinnamomum camphora* as set forth in SEQ ID NO: 40. In another embodiment
- 35

the thioesterase has at least 60% homology to the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42.

In one embodiment, the yeast cell expresses:

- 5 i) at least one heterologous $\Delta 10$ desaturase; and
- ii) at least one heterologous FAR; and
- iii) optionally overexpresses an acetyltransferase, and
- iv) optionally overexpresses a thioesterase,

wherein the $\Delta 10$ desaturase has a higher specificity towards tetradecanoyl-CoA than
 10 towards hexadecanoyl-CoA and/or wherein the fatty acyl-CoA reductase has a higher
 specificity towards desaturated tetradecanoyl-CoA than towards desaturated
 hexadecanoyl-CoA, whereby the yeast cell produces a fatty alcohol having a carbon
 chain length of 14 and desaturated in position 10. The acetyltransferase may be the
 AcT of SEQ ID NO: 21 (Atf1, the *S. cerevisiae* AcT) or a variant thereof having at least
 15 75% homology to Sc_Atf1, such as at least 76%, such as at least 77%, such as at least
 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at
 least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as
 at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such
 as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%,
 20 such as at least 94%, such as at least 95%, such as at least 96%, such as at least
 97%, such as at least 98%, such as at least 99%, such as 100% homology to SEQ ID
 NO: 21. In some embodiments, the thioesterase has at least 60% homology to a
 thioesterase selected from the thioesterase derived from *Cuphea palustris* as set forth
 in SEQ ID NO: 23, the thioesterase from *Cuphea hookeriana* as set forth in SEQ ID
 25 NO: 38, the thioesterase from *Cinnamomum camphora* as set forth in SEQ ID NO: 40,
 and the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 42. Preferably,
 the thioesterase has at least 60% homology to the thioesterase derived from
Cinnamomum camphora as set forth in SEQ ID NO: 40 or from *Escherichia coli* as set
 forth in SEQ ID NO: 42. In one embodiment, the thioesterase has at least 60%
 30 homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ
 ID NO: 40. In another embodiment the thioesterase has at least 60% homology to the
 thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42.

In one embodiment, the yeast cell expresses:

- 35 i) at least one heterologous $\Delta 11$ desaturase; and
- ii) at least one heterologous FAR; and

- iii) optionally overexpresses an acetyltransferase, and
- iv) optionally overexpresses a thioesterase,

wherein the $\Delta 11$ desaturase has a higher specificity towards tetradecanoyl-CoA than towards hexadecanoyl-CoA and/or wherein the fatty acyl-CoA reductase has a higher specificity towards desaturated tetradecanoyl-CoA than towards desaturated hexadecanoyl-CoA, whereby the yeast cell produces a fatty alcohol having a carbon chain length of 14 and desaturated in position 11. The acetyltransferase may be the AcT of SEQ ID NO: 21 (Atf1, the *S. cerevisiae* AcT) or a variant thereof having at least 75% homology to Sc_At1, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to SEQ ID NO: 21. In some embodiments, the thioesterase has at least 60% homology to a thioesterase selected from the thioesterase derived from *Cuphea palustris* as set forth in SEQ ID NO: 23, the thioesterase from *Cuphea hookeriana* as set forth in SEQ ID NO: 38, the thioesterase from *Cinnamomum camphora* as set forth in SEQ ID NO: 40, and the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 42. Preferably, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40 or from *Escherichia coli* as set forth in SEQ ID NO: 42. In one embodiment, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40. In another embodiment the thioesterase has at least 60% homology to the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42.

In one embodiment, the yeast cell expresses:

- i) at least one heterologous $\Delta 12$ desaturase; and
- ii) at least one heterologous FAR; and
- iii) optionally overexpresses an acetyltransferase, and
- iv) optionally overexpresses a thioesterase,

wherein the $\Delta 12$ desaturase has a higher specificity towards tetradecanoyl-CoA than towards hexadecanoyl-CoA and/or wherein the fatty acyl-CoA reductase has a higher specificity towards desaturated tetradecanoyl-CoA than towards desaturated hexadecanoyl-CoA, whereby the yeast cell produces a fatty alcohol having a carbon

chain length of 14 and desaturated in position 12. The acetyltransferase may be the AcT of SEQ ID NO: 21 (Atf1, the *S. cerevisiae* AcT) or a variant thereof having at least 75% homology to Sc_At1, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%, such as 100% homology to SEQ ID NO: 21. In some embodiments, the thioesterase has at least 60% homology to a thioesterase selected from the thioesterase derived from *Cuphea palustris* as set forth in SEQ ID NO: 23, the thioesterase from *Cuphea hookeriana* as set forth in SEQ ID NO: 38, the thioesterase from *Cinnamomum camphora* as set forth in SEQ ID NO: 40, and the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 42. Preferably, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40 or from *Escherichia coli* as set forth in SEQ ID NO: 42. In one embodiment, the thioesterase has at least 60% homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ ID NO: 40. In another embodiment the thioesterase has at least 60% homology to the thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42.

In one embodiment, the yeast cell expresses:

- i) at least one heterologous $\Delta 13$ desaturase; and
- ii) at least one heterologous FAR; and
- iii) optionally overexpresses an acetyltransferase, and
- iv) optionally overexpresses a thioesterase,

wherein the $\Delta 13$ desaturase has a higher specificity towards tetradecanoyl-CoA than towards hexadecanoyl-CoA and/or wherein the fatty acyl-CoA reductase has a higher specificity towards desaturated tetradecanoyl-CoA than towards desaturated hexadecanoyl-CoA, whereby the yeast cell produces a fatty alcohol having a carbon chain length of 14 and desaturated in position 13. The acetyltransferase may be the AcT of SEQ ID NO: 21 (Atf1, the *S. cerevisiae* AcT) or a variant thereof having at least 75% homology to Sc_At1, such as at least 76%, such as at least 77%, such as at least 78%, such as at least 79%, such as at least 80%, such as at least 81%, such as at least 82%, such as at least 83%, such as at least 84%, such as at least 85%, such as at least 86%, such as at least 87%, such as at least 88%, such as at least 89%, such

as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%,
such as at least 94%, such as at least 95%, such as at least 96%, such as at least
97%, such as at least 98%, such as at least 99%, such as 100% homology to SEQ ID
NO: 21. In some embodiments, the thioesterase has at least 60% homology to a
5 thioesterase selected from the thioesterase derived from *Cuphea palustris* as set forth
in SEQ ID NO: 23, the thioesterase from *Cuphea hookeriana* as set forth in SEQ ID
NO: 38, the thioesterase from *Cinnamomum camphora* as set forth in SEQ ID NO: 40,
and the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 42. Preferably,
the thioesterase has at least 60% homology to the thioesterase derived from
10 *Cinnamomum camphora* as set forth in SEQ ID NO: 40 or from *Escherichia coli* as set
forth in SEQ ID NO: 42. In one embodiment, the thioesterase has at least 60%
homology to the thioesterase derived from *Cinnamomum camphora* as set forth in SEQ
ID NO: 40. In another embodiment the thioesterase has at least 60% homology to the
thioesterase derived from *Escherichia coli* as set forth in SEQ ID NO: 42.

15 In some embodiments, the yeast cell further has one or more mutations resulting in a
partial or total loss of activity of one or more lipases, as detailed herein above. In some
embodiments, the yeast cell further has a mutation resulting in modification of one or
more subunits of the fatty acyl synthase complex; particularly mutations resulting in
20 modifications of the ketone synthase domain, as detailed herein above, are
contemplated.

In some embodiments, the yeast cell further has one or more mutations resulting in a
partial or total loss of activity of one or more lipases and a mutation in one or more
25 modification of one or more subunits of the fatty acyl synthase complex, particularly
mutations resulting in modifications of the ketone synthase domain, as described
herein above.

Nucleic acids

It will be understood that throughout the present disclosure, the term 'nucleic acid
30 encoding an activity' shall refer to a nucleic acid molecule capable of encoding a
peptide, a protein or a fragment thereof having said activity. Such nucleic acid
molecules may be open reading frames or genes or fragments thereof. The nucleic
acid construct may also be a group of nucleic acid molecules, which together may
encode several peptides, proteins or fragments thereof having an activity of interest.

35 The term 'activity' or 'activity of interest' refers to one of the following activities: a

desaturase as described herein, a fatty acyl-CoA reductase, an aldehyde-forming fatty acyl coA reductase, a thioesterase and/or an acetyltransferase activity. The nature of the one or more activity of interest will depend on the nature of the desired product one wishes to obtain with the present methods.

5

In some embodiments of the present methods, each of the nucleic acids encoding each of the present activities, i.e. a desaturase as described herein, a fatty acyl-CoA reductase, an aldehyde-forming fatty acyl-CoA reductase, a thioesterase and/or an acetyltransferase, may be comprised within the genome of the yeast cell or within a vector comprised within yeast cell.

10

In some embodiments, each of the nucleic acids encoding each of the present activities may be present in the genome of said yeast cell, either because the nucleic acid encodes a native protein, or because it has been integrated therein by genome engineering or genome editing or by crossing yeast cells of different mating types. Methods for integrating a nucleic acid are well known in the art. Thus in some embodiments the activity of interest is encoded by introduction of a heterologous nucleic acid in the yeast cell. The heterologous nucleic acid encoding said activity may be codon-optimised, or may comprise features that can help improve the activity. For example, the heterologous nucleic acid may be modified so as to encode a modified protein. Such modifications include, but are not limited to, the introduction of localisation signals, gain-of-function or loss-of-function mutations, fusion of the protein to a marker or a tag such as fluorescent tag, insertion of an inducible promoter, introduction of modifications conferring increased stability and/or half-life.

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The introduction of the heterologous nucleic acid encoding the activity of interest can be performed by methods known in the art. The skilled person will recognise that such methods include, but are not limited to: cloning and homologous recombination-based methods. Cloning methods may involve the design and construction of a plasmid in an organism such as *Escherichia coli*. The plasmid may be an integrative or a non-integrative vector. Cloning-free methods comprise homologous recombination-based methods such as adaptamer-mediated PCR or gap repair. Such methods often result in integration of the heterologous nucleic acid in the genome of the yeast cell.

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The nucleic acids encoding the activities of interest may be present in high copy number.

Methods for production of desaturated fatty alcohols and/or desaturated fatty acyl acetates

Provided herein is a method for production of a desaturated fatty acid and optionally a
5 desaturated fatty acyl acetate in a yeast cell, said method comprising the steps of
providing a yeast cell and incubating said yeast cell in a medium, wherein the yeast cell
expresses:

- 10 i) at least one heterologous desaturase capable of introducing at least one
double bond in a fatty acyl-CoA having a carbon chain length of 14, thereby
converting at least part of said fatty acyl-CoA to a desaturated fatty acyl-
CoA; and
- ii) at least one heterologous fatty acyl-CoA reductase, capable of converting at
least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol,
thereby producing said desaturated fatty alcohol; and
- 15 iii) optionally an acetyltransferase capable of converting at least part of said
desaturated fatty alcohol to a desaturated fatty acyl acetate, thereby
producing said desaturated fatty acyl acetate;

wherein the desaturase has a higher specificity towards tetradecanoyl-CoA than
towards hexadecanoyl-CoA and/or wherein the fatty acyl-CoA reductase has a higher
20 specificity towards desaturated tetradecanoyl-CoA than towards desaturated
hexadecanoyl-CoA.

The yeast cell may be able to synthesise tetradecanoyl-CoA naturally or may be
engineered to synthesise tetradecanoyl-CoA or tetradecanoyl-CoA may be provided in
25 the medium in which the cell is incubated, as described in the section "fatty acyl-CoA".
The at least one heterologous desaturase and at least one heterologous fatty acyl-CoA
reductase may be as described herein elsewhere. The yeast cell may be as described
above.

30 The yeast cells described herein can be used in a method for producing a desaturated
fatty alcohol and/or a desaturated fatty acyl acetate having a chain length of 14 with
unprecedented titres.

In particular, in some embodiments, the ratio of desaturated tetradecanoyl-CoA to
35 desaturated hexadecanoyl-CoA is of at least 0.1, such as at least 0.2, such as at least

0.3, such as at least 0.4, such as at least 0.5, such as at least 0.75, such as at least 1, such as at least 2, such as at least 3, such as at least 4, such as at least 5, such as at least 6, such as at least 7, such as at least 8, such as at least 9, such as at least 10, such as at least 12.5, such as at least 15, or more.

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In some embodiments, the method yields a titre of desaturated fatty alcohols of at least 1 mg/L, such as at least 1.5 mg/L, such as at least 5 mg/L, such as at least 10 mg/L, such as at least 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 750 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, or more.

10

In some embodiments, the method yields a titre of desaturated fatty alcohol having a chain length of 14 of at least 1 mg/L, such as at least 1.5 mg/L, such as at least 5 mg/L, such as at least 10 mg/L, such as at least 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 750 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, or more.

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In some embodiments, the method yields desaturated fatty alcohols comprising at least 1% of a desaturated fatty alcohol having a chain length of 14, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 4.5%, such as at least 5%, such as at least 7.5%, such as at least 10%, or more.

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In some embodiments, the method yields a titre of desaturated fatty acetates of at least 1 mg/L, such as at least 1.5 mg/L, such as at least 5 mg/L, such as at least 10 mg/L, such as at least 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 750 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, or more.

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In some embodiments, the method yields a titre of desaturated fatty acetate having a chain length of 14 of at least 1 mg/L, such as at least 1.5 mg/L, such as at least 5 mg/L, such as at least 10 mg/L, such as at least 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at

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least 750 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, or more.

5 In some embodiments, the method yields desaturated fatty acetates comprising at least 1% of a desaturated fatty acetate having a chain length of 14, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 4.5%, such as at least 5%, such as at least 7.5%, such as at least 10%.

10 In some embodiments, the yeast cell may further express an aldehyde-forming fatty acyl-CoA reductase EC 1.2.1 .50 (FAR') as described herein above.

Recovery

15 It may be desirable to recover the products obtained by the methods disclosed herein. Thus the present methods may comprise a further step of recovering the desaturated fatty alcohol and/or the desaturated fatty acyl acetate produced by the present yeast cell.

20 In some embodiments, the method comprises a step of recovering the desaturated fatty alcohols. In a particular embodiment, the method comprises a step of recovering the desaturated fatty alcohols having a carbon chain length of 14. In other embodiments, the method comprises a step of recovering the fatty acyl acetates. In a particular embodiment, the method comprises a step of recovering the fatty acyl acetates having a carbon chain length of 14.

25 Methods for recovering the products obtained by the present invention are known in the art and may comprise an extraction with a hydrophobic solvent such as decane, hexane or a vegetable oil.

30 The recovered products may be modified further, for example desaturated fatty alcohols may be converted to the corresponding desaturated fatty aldehydes as described herein above.

35 The recovered products, i.e. the desaturated fatty alcohols and/or desaturated fatty acyl acetates, may also be formulated into a pheromone composition. The composition

may further comprise one or more additional compounds such as a liquid or solid carrier or substrate. Fatty aldehydes obtained from said desaturated fatty alcohols may also be comprised in such compositions.

5 *Kit*

Provided herein is a kit of parts for performing the present methods. The kit of parts may comprise a yeast cell "ready to use" as described herein. In one embodiment, the yeast cell is a *Yarrowia* cell, such as a *Yarrowia lipolytica* cell.

10 In another embodiment, the kit of parts comprises a nucleic acid construct encoding the activities of interest to be introduced in the yeast cell. The nucleic acid construct may be provided as a plurality of nucleic acid constructs, such as a plurality of vectors, wherein each vector encodes one or several of the desired activities.

15 The kit of parts may optionally comprise the yeast cell to be modified.

In some embodiments, the kit of parts comprises all of the above.

Pheromone composition

20 The present disclosure thus provides compounds, in particular fatty alcohols and fatty acyl acetates, as well as derivatives thereof, and their use. In particular, the compounds obtainable using the present cells and methods are useful as components of pheromone compositions. Such pheromone compositions may be useful for integrated pest management. They can be used as is known in the art for e.g. mating
25 disruption.

The desaturated fatty alcohols and desaturated fatty acyl acetates obtainable by the present methods or using the present yeast cells may be formulated in a pheromone composition.

30

Such pheromone compositions may be used as integrated pest management products, which can be used in a method of monitoring the presence of pest or in a method of disrupting the mating of pest.

Pheromone compositions as disclosed herein may be used as biopesticides. Such compositions can be sprayed or dispensed on a culture, in a field or in an orchard. They can also, as is known in the art, be soaked e.g. onto a rubber septa, or mixed with other components. This can result in mating disruption, thereby preventing pest reproduction, or it can be used in combination with a trapping device to entrap the pests. Non-limiting examples of pests against which the present pheromone compositions can be used are: cotton bollworm (*Helicoverpa armigera*), striped stemborer (*Chilo suppressalis*), diamond back moth (*Plutella xylostella*), cabbage moth (*Mamestra brassicae*), large cabbage-heart caterpillar (*Crocidolomia binotalis*), European corn stalk borer (*Sesamia nonagrioides*), currant clearwing (*Synanthedon tipuliformis*) and artichoke plume moth (*Platyptilia carduidactyla*). Accordingly, use of the present compositions on a culture can lead to increased crop yield, with substantially no environmental impact.

The relative amounts of fatty alcohols and fatty acyl acetates in the present pheromone compositions may vary depending on the nature of the crop and/or of the pest to be controlled; geographical variations may also exist. Determining the optimal relative amounts may thus require routine optimisation. The pheromone compositions may also comprise fatty aldehydes.

Examples of compositions used as repellents can be found in Kehat & Dunkelblum, 1993, for *H. armigera*, in Alfaro et al., 2009, for *C. suppressalis*, in Eizaguirre et al., 2002, for *S. nonagrioides*; in Wu et al., 2012, for *P. xylostella*; in Bah et al., 2003, for *P. carduidactyla*.

In some embodiments, the pheromone composition may further comprise one or more additional compounds such as a liquid or solid carrier or substrate. For example, suitable carriers or substrate include vegetable oils, refined mineral oils or fractions thereof, rubbers, plastics, silica, diatomaceous earth, wax matrix and cellulose powder.

The pheromone composition may be formulated as is known in the art. For example, it may be in the form of a solution, a gel, a powder. The pheromone composition may be formulated so that it can be easily dispensed, as is known in the art.

Examples

Example 1: Construction of plasmids and strains

Genes encoding desaturases from *Pelargonium hortorum* (SEQ ID NO: 1) and *Ricinus communis* (SEQ ID NO: 3) were synthesized by GeneArt (Life Technologies) in codon-optimized versions for *Y. lipolytica*. The genes encoding desaturases from *Amyelois transitella* (SEQ ID NO: 5 and SEQ ID NO: 7), from *Drosophila melanogaster* (SEQ ID NO: 9), and *OLE1* from *S. cerevisiae* were synthesized by GeneArt in codon-optimized version for *S. cerevisiae*. The synthetic genes encoding *Amyelois transitella* desaturase and *S. cerevisiae* desaturase *OLE1* had attB1 -attB2 sites incorporated, which allowed to clone these genes into the vector pDONR 221 via Gateway cloning system (Invitrogen: Gateway® Technology Manual. [http://tools.invitrogen.com/content/sfs/manuals/gatewayman.pdf]. The gene encoding alcohol acetyltransferase *ATF1* (SEQ ID NO: 19) was amplified from genomic DNA preparation of *S. cerevisiae* strain CEN.PK102-5B. A gene encoding fatty acyl reductase from *Helicoverpa armigera* was modified so that its putative native KKS YE signal was replaced with HDEL signal from *S. cerevisiae* and this gene was also synthesized by GeneArt (Life Technologies) in codon-optimized version for *S. cerevisiae*. All the genes were amplified by PCR to obtain the fragments for cloning into yeast expression vectors. The primers are listed in Table 1 and the resulting DNA fragments are listed in Table 2. The PCR products were separated on a 1%-agarose gel containing RedSafe™ (iNtRON Biotechnology). PCR products of the correct size were excised from the gel and purified using the Nucleospin® Gel and PCR Clean-up kit (Macherey-Nagel).

Table 1: Primers.

Primer name	Primer sequence, 5'→3'	SEQ ID NO:
PR-1 852 (PTDH3_fw)	CACGCGAUATAAAAAACACGCTTTTT CAG	46
PR-1 853 (PTDH3_rv)	ACCTGCACUTTTGTTTGTTTATGTGTG TTTATTC	47
PR-1 565 (PTEF1)	ATGACAGAUTTGTAAATTAACCTTAG	48
PR-8332 (Har_FAR_U1_fw)	AGTGCAGGUAAAACAATGGTTGTCTT GACCTCCAAAG	49

PR-1 0739 (Har_FAR_HDEL_U1_rev)	cgtagcgaUttacaattcatcatgtccaagaaatgtcta acac	50
PR-14318 (Phd9_U2_fw)	ATCTGTCAUAAAACAatggcgctcctgtgaa c	51
PR-14276 (Phd9_U2_rev)	cacgcgaUttagacctttcgg	52
PR-14319 (RCd9_U2_fw)	ATCTGTCAUAAAACAatggccctgaag	53
PR-14278 (RCd9_U2_rev)	cacgcgaUttacagcttcacctg	54
PR-14320 (Atf1_U2_fw)	ATCTGTCAUAAAACAATGAATGAAATC GATGAG	55
PR-14321 (Atf1_U2_rev)	CACGCGAUCTAAGGGCCTAAAAGGA GAGC TTTG	56
PR-1 5974 (Dmd9_U1_fw)	AGTGCAGGUAAAACAatggctccatactctag aatc	57
PR-1 5975 (Dmd9_U1_rev)	CGTGCGAUttatctggactgtcaacc	58
PR-1 5976 (attB1_Dmd9_F)	GGGGACAAGTTTGTACAAAAAAGCAGGCT ATGGCTCCATACTCTAGAATCTAC	59
PR-1 5977 (attB2_Dmd9_R)	GGGGACCACTTTGTACAAGAAAGCTGGGT TTATCTGGACTTGTCAACCAACAAAACGTT TCTAG	60
PR-1 5978 (attB1_Ph9_F)	GGGGACAAGTTTGTACAAAAAAGCAGGCT ATGGCCCTGAAGCTGAACCCCTTC	61
PR-1 5979 (attB2_Ph9_R)	GGGGACCACTTTGTACAAGAAAGCTGGGT TTACAGCTTCACCTGTCGGTCAAG	62
PR-1 5980 (attB1_Rcd9_F)	GGGGACAAGTTTGTACAAAAAAGCAGGCT ATGGGCGTCCTGCTGAACATCTG	63
PR-1 5981 (attB1_Rcd9_R)	GGGGACCACTTTGTACAAGAAAGCTGGGT TTAGACCTTTCGGTCAAGATCCA	64

Table 2: DNA fragments obtained by PCR using the indicated template and primers.

DNA fragment ID and name	Description	Fw primer	Rv primer	Template DNA
BB0410	PTDH3 promotor from	PR-	PR-1853	Genomic DNA

(PTDH3)	<i>S. cerevisiae</i>	1852 (PTDH3_fw)	(PTDH3_rv)	from <i>S. cerevisiae</i> CEN.PK102-5B
BB0464 (<-PTDH3-PTEF1->)	PTDH3 and PTEF1 promoter from <i>S. cerevisiae</i>	PR-1565 (PTEF1)	PR-1853 (PTDH3_rv)	Genomic DNA from <i>S. cerevisiae</i> CEN.PK102-5B
BB0915 (HAR_FAR_HDEL<-)	Fatty acyl-CoA reductase from <i>Helicoverpa armigera</i> with modified C-terminus	PR-8332 (HAR_FAR_JJ1_fw)	PR-10739 (HAR_FAR_HDEL_U1_rev)	
BB1420 (Phd9)	Desaturase from <i>Pelargonium hortorum</i>	PR-14318 (Phd9_U2_fw)	PR-14276 (Phd9_U2_rev)	
BB1421 (RCd9)	Desaturase from <i>Ricinus communis</i>	PR-14319 (RCd9_U2_fw)	PR-14278 (RCd9_U2_rev)	
BB1422 (Atf1->)	Alcohol acetyltransferase from <i>Saccharomyces cerevisiae</i>	PR-14320 (Atf1_U2_fw)	PR-14321 (Atf1_U2_rev)	Genomic DNA from <i>S. cerevisiae</i> CEN.PK102-5B
BB1696 (Dmd9)	Desaturase from <i>Drosophila melanogaster</i>	PR-15974 (Dmd9_U1_fw)	PR-15975 (Dmd9_U1_rev)	
BB1870 (Dmd9)	Desaturase from <i>Drosophila melanogaster</i>	PR-15976 (attB1_Dmd9_F)	PR-15977 (attB2_Dmd9_R)	pCfB5316

BB1 871 (Phd9)	Desaturase from <i>Pelargonium hortorum</i>	PR-15978 (attB1_ Phd9_ F)	PR-15979 (attB2_Ph9_R)	pCfB4584
BB1 872 (Rcd9)	Desaturase from <i>Ricinus communis</i>	PR-15980 (attB1_ Rcd9_ F)	PR-15981 (attB1_Rcd9_R)	pCfB4585

Example 2: Cloning of vectors p YEX-CHT-Dmd9, pYEX-CHT-Phd9, pYEX-CHT-Rcd9, pYEX-CHT-Atrd1432, pYEX-CHT-Atrd236 and pYEX-CHT-OLE1

- DNA fragments BB1870, BB1 871, and BB1 872 were amplified from respectively
- 5 plasmids pCfB531 6, pCfB4584 and pCfB4585 by using the Maxima Hot Start Green PCR Master Mix (2X) (ThermoFisher Scientific) according to the manufacturers protocol. The PCR mix contained 20 μ l water, 25 μ l Maxima Hot Start Green PCR Master Mix, 2.5 μ l forward primer (10 μ M), 2.5 μ l reverse primer (10 μ M) and 5ng DNA
- 10 template and the following PCR program was used: 94°C for 2 min, 35 cycles of [94°C for 15 sec, 55°C for 30 sec, 72°C for 2 min 30 sec], 72°C for 7 min. The PCR products were separated on a 1%-agarose gel. PCR products of the correct size were excised from the gel and purified using the Nucleospin® Gel and PCR Clean-up kit (Macherey-Nagel).
- 15 The resulting DNA fragments (BB1 870, BB1 871 and BB1 872) were cloned into the vector pDONR 221 by Gateway cloning technology creating the so called "entry clones" (ThermoFisher Scientific). The BP reaction was performed by mixing 100 ng of synthetic genes, 100 ng of pDONR 221 and 1 μ l of BP clonase (Life Technologies). The reaction was incubated at room temperature for 1 hour. The reaction mix was
- 20 transformed into *E. coli* competent HB101 cells (Life Technologies) by heat shock and the cells were plated on Lysogeny Broth (LB) agar plates with 50 mg/L kanamycin and incubated overnight at 37°C. Single colonies were inoculated into 5 ml of liquid LB with 50 mg/L kanamycin in 13-ml sterile tubes and cultivated with shaking overnight. The plasmids were purified from overnight *E. coli* cultures and sequenced to confirm correct
- 25 cloning. The genes were shuttled from the entry clones to the destination yeast

expression vector pYEX-CHT-DEST (Ding BJ, Carraher C, Lofstedt C. 2016. Sequence variation determining stereochemistry of a $\Delta 11$ desaturase active in moth sex pheromone biosynthesis. *Insect Biochem Mol Biol.* 74: 68-75. doi: 10.1016/j.ibmb.2016.05.002.) by mixing 100 ng of the entry clones with 100 ng of destination vector pYEX-CHT-DEST and 1 μ L of LR clonase (Invitrogen). The reaction was incubated at room temperature for 1 hour, followed by transformation into *E. coli* competent HB101 cells by heat-shock. The cells were plated on Lysogeny Broth (LB) agar plates with 100 mg/L ampicillin. The plasmids were purified from overnight *E. coli* cultures and the correct cloning was confirmed by sequencing.

Example 3: Cloning of vectors pCfB5316, pCfB4584, pCfB4585, pCfB4580

The DNA biobricks BB0410, BB1696, BB0301, BB1420, BB0301, BB1421, BB0464, BB0915 and BB1422 were amplified by PCR like following. The PCR mix contained 32 μ L water, 10 μ L high fidelity Phusion® polymerase buffer (5x), 1 μ L dNTPs (10mM), 1 μ L Phusion U polymerase, 2.5 μ L forward primer (10 μ M), 2.5 μ L reverse primer (10 μ M) and 1 μ L DNA template and the following PCR program was used: 94°C for 2 min, 30 cycles of [94°C for 15 sec, 52°C for 20 sec, 68°C for 1 min 30 sec], 68°C for 2 min, pause at 10°C.

The integrative vector EasyClone 2.0 pCfB2909 (XII-5-MarkerFree) is described in Jessop-Fabre et. al., 2016 and pCfB2190 is described in Stovicek et al., 2015. Plasmid pCfB2912 was constructed by USER fusion of DNA fragments BB0593 (contains pCfB387 vector backbone) and BB0598 (contains nourseothricin resistance cassette), as described in Stovicek et al, 2015. All integrative vectors were linearized with FastDigest® AsiSI (Fermentas) for 2 hours at 37°C and then nicked with Nb.BsmI (New England Biolabs) for 1 hour at 65°. The resulting vectors containing sticky ends were separated by gel electrophoresis, excised and gel-purified using the Nucleospin® Gel and PCR Clean-up kit (Macherey-Nagel). The DNA fragments were cloned into the so prepared vectors by USER-cloning via the following protocol: 1 μ L of linearized plasmid, 1 μ L of promoter fragment, 1.5 μ L of gene fragment, 1 μ L high fidelity Phusion® polymerase buffer (5x), and 0.5 μ L USER enzyme (New England Biolabs) were mixed and incubated at 37°C for 25 min and at 25°C for 25 min. The reaction was transformed into chemically competent *E. coli* DHalpha cells and the cells were plated on Lysogeny Broth (LB) agar plates with 100 mg/L ampicillin. The plates were incubated overnight at 37°C and the resulting colonies were screened by colony PCR.

The plasmids were purified from overnight *E. coli* cultures and the correct cloning was confirmed by sequencing. The constructed vectors are listed in Table 3.

Table 3: Expression vectors.

Expression vector name	Selection marker	Parent vector	DNA fragments cloned into parent vector
pYEX-CHT-Dmd9	Ura, Leu	pYEX-CHT-DEST	BB1 870 (Dmd9)
pYEX-CHT-Phd9	Ura, Leu	pYEX-CHT-DEST	BB1 871 (Phd9)
pYEX-CHT-Rcd9	Ura, Leu	pYEX-CHT-DEST	BB1 872 (Rcd9)
pYEX-CHT-Atrd1432	Ura, Leu	pYEX-CHT-DEST	
pYEX-CHT-Atrd236	Ura, Leu	pYEX-CHT-DEST	
pYEX-CHT-OLE1	Ura, Leu	pYEX-CHT-DEST	
pCfB4584 (pXI-5-loxP-NatMXsyn->PTEF1 -Phd9)	NatMXSyn	pCfB2912	BB0301 (PTEF1->), BB1420 (Phd9->)
pCfB4585 (pXI-5-loxP-NatMXsyn->PTEF1 -Rcd9)	NatMXSyn	pCfB2912	BB0301 (PTEF1->), BB1421 (Rcd9->)
pCfB4580 (pXI-KILeu2syn-Har_FAR_HDEL_PTDH3<~>PTef1 -Atf1)	KILEU2	pCfB21 90	BB0464 (<-PTDH3-PTEF1 ->), BB091 5 (HAR_FAR_HDEL<-), BB1422 (Atf1->)
pCfB5316 (pXII-5-Dmd9-PTDH3<-)	markerfree	pCfB2909	BB041 0 (PTDH3<-), BB1 696 (Dmd9<-)

Example 4: Construction of strains

The pYEX-CHT derived recombinant expression vectors containing the different desaturase genes were introduced into *S. cerevisiae* deficient for both *OLE1* and *EL01* (MATa elol ::HIS3 olel ::LEU2 ade2 his3 leu2 ura3; (Schneider et al., 2000)), using the S.c. easy yeast transformation kit (Life Technologies). For selection of uracil and leucine prototrophic clones, the transformed yeast cells were plated on medium composed of 0.7% YNB (without amino acid, with ammonium sulfate), 1.546% drop-out mix lacking uracil and leucine (Formedium™ LTD, Norwich, England), 2% glucose, 1% tergitol (type Nonidet NP-40, Sigma-Aldrich Sweden AB, Stockholm, Sweden), 0.01% adenine (Sigma), and 0.5 mM oleic acid (Sigma). The constructed yeast strains are listed in Table 4.

The integrative expression vectors pCfB4580 and pCfB531 6 were linearized with FastDigest® NotI (Fermentas). pCfB4580 was transformed into *S. cerevisiae* CEN.PK1 02-5B using lithium-acetate protocol (Gietz & Schiestl, 2007) leading to strain ST4854. Positive transformants were selected on yeast synthetic drop-out plates without leucine (Sigma-Aldrich). Correct integration of the expression constructs into the genome of *S. cerevisiae* was confirmed by colony PCR. Strain ST5290 was constructed by integrating pCfB531 6 into ST4854 using a method described in (Jessop-Fabre et al., 2016). The constructed strains are listed in Table 5.

Example 5: A9 desaturases activities and specificities

The activities and specificities of desaturases were tested in a *S. cerevisiae* strain with deletions of *OLE1* and *EL01* genes, encoding for A9-fatty acid desaturase and medium-chain acyl elongase respectively (Schneider et al., 2000).

Three individual colonies of strains ST_Atr1432, ST_Atr236, ST_PhD9, ST_Rcd9, ST_ScOLE1 and ST_DmeD9 were inoculated into 1 mL selective media (SC-Ura-Leu) and incubated at 30°C and 300 rpm for 48 h. The cultures were diluted to an OD600 of 0.4 in 5 mL selective medium supplemented with 2 mM CuSO₄ and the 0.5 mM methyl myristate (14:Me) (Larodan Fine Chemicals, Sweden). The methyl myristate stock solution was prepared to a concentration of 100 mM in 96% ethanol. The yeast cultures were incubated at 30°C at 300 rpm for 48 hours.

1 mL of culture was sampled and 3.12 µg of nonadecylic acid methyl ester was added as internal standard. Total lipids were extracted using 3.75 mL of methanol/chloroform

- (2:1, v/v), in a glass vial. One ml_l of acetic acid (0.1 M) and 1.25 ml_l of water were added to the tube. Tubes were vortexed vigorously and centrifuged at 2,000xg for 2 min. The bottom chloroform phase, about 1 ml_l, containing the total lipids, was transferred to a new glass vial and the solvent was evaporated to dryness. Fatty acid methyl esters (FAMES) were made from this total lipid extract by acid methanolysis. One ml_l of 2% sulfuric acid in methanol (v/v) was added to the tube, vortexed vigorously, and incubated at 90°C for 1 h. After incubation, 1 ml_l of water was added and mixed well, and then 1 ml_l of hexane was used to extract the FAMES.
- The methyl ester samples were subjected to GC-MS analyses on a Hewlett Packard 6890 GC coupled to a mass selective detector HP 5973. The GC was equipped with an INNOWax column (30 m x 0.25 mm x 0.25 μm), and helium was used as the carrier gas (average velocity: 33 cm/s). The MS was operated in electron impact mode (70 eV), and the injector was configured in splitless mode at 220°C. The oven temperature was set to 80°C for 1 min, then increased at a rate of 10°C/min up to 210°C, followed by a hold at 210°C for 15 min, and then increased at a rate of 10°C/min up to 230°C followed by a hold at 230°C for 20 min. The monounsaturated fatty-acid products were identified by comparing their retention times and mass spectra with those of synthetic standards. Data were analyzed by the ChemStation software (Agilent, Technologies, USA).

The measured concentrations of Z9-14:Me and Z9-16:Me (Table 4) show that strain ST_DmeD9, expressing desaturase from *D. melanogaster*, resulted in the highest concentration of Z9-14:Me (3.67 mg/L) and in the maximal ratio of Z9-14:Me and Z9-16:Me. This indicates that among the tested desaturases, *D. melanogaster* desaturase has the highest activity and specificity towards C14-CoA substrate.

Table 4: Activity and specificity of heterologous desaturases in yeast.

Strain name	Over-expressed desaturase	Parent strain (key characteristics)	Vectors introduced into parent strain	Z9-14:Me (mg/L)	Z9-16:Me (mg/L)	Ratio of 14:1/16:1 [specificity]
ST_Atr1432	Atr1432	<i>Δole1Δelo1</i>	pYEX-CHT-Atrd1432	0.25	0.56	0.45

ST_Atr236	Atr236	$\Delta ole1 \Delta elo1$	pYEX-CHT-Atr236	0.03	0.13	0.20
ST_Ph9	Ph9	$\Delta ole1 \Delta elo1$	pYEX-CHT-Ph9	0.00	0.00	0.00
ST_Rcd9	Rcd9	$\Delta ole1 \Delta elo1$	pYEX-CHT-Rcd9	0.00	0.00	0.00
ST_ScOLE1	ScOLE1	$\Delta ole1 \Delta elo1$	pYEX-CHT-OLE1	0.39	3.01	0.13
ST_DmeD9	Dme Δ 9	$\Delta ole1 \Delta elo1$	pYEX-CHT-Dmd9	3.67	0.24	15.29

Example 6: Production of (Z)9-tetradecen-1-yl acetate

The strains for production of pheromone were created on the basis of *S. cerevisiae* CEN.PK102-5B, which had active *OLE1* and *EL01* genes. The obtained strains are listed in table 5.

Strains ST4854 and ST5290 were inoculated into 5 ml synthetic complete medium (lacking histidine, leucine, tryptophan supplemented with 20 mg/L uracil and 76 mg/L histidine) and cultivated in 12-ml glass tubes (Duran, Wertheim, Germany) with metal labocap lids (Ludiswiss, Flawil, Switzerland) overnight at 30 °C with shaking at 250 rpm. The following day the overnight culture was centrifuged, the supernatant was discarded and the pellet was resuspended in 2 ml of mineral medium, which had the composition as described in (Jensen et al, 2014). The medium was supplemented with 76 mg/L histidine and 20 mg/L uracil. The cultures were incubated at 30 °C with shaking at 250 rpm for 48 hours.

1 mL sample of culture was transferred into a 4-mL glass vial and 10 μ l of internal standard stock (1 μ g/ μ l (Z)10-heptan-1-yl methyl ester in 100% ethanol) was added. The vials were covered with small pieces of aluminum foil and we used a needle to pierce small holes in the foil covers. The samples were vortexed and placed at -80 °C for storage until analysis. The samples were freeze-dried (Freezone6 and Stoppering tray dryer, Labconco, Kansas City, USA) at -40 °C, then 1 mL chloroform :methanol 2:1 was added to disrupt the cells. The mix was vortexed for 45 s and left at room temperature for 4 hours. The organic solvents were evaporated slowly under a nitrogen stream. 1 ml of hexane was added, the samples were vortexed for 10 s, centrifuged

and 200 μ l were transferred to a new glass vial. GC-MS analysis was performed as described in Example 5. The concentration of (Z)-9-tetradecen-1-yl acetate was calculated based on internal standard.

- 5 As apparent from the results, overexpression of *D. melanogaster* desaturase increased the titer of Z9:14:OAc more than 5-fold. Moreover, the product fraction of the total fatty acyl acetates increased from 2 to 10%.

Table 5: Production of (Z)-9-tetradecen-1 -yl acetate by yeast

Strain	Overex-pressed genes	Parent strain	Vectors introduced into parent strain	29-14:OAc (mg/L)	% of Z9-14:OAc in relation to total fatty acyl acetates
ST4854	<i>ATF1</i> from <i>S. cerevisiae</i> , <i>Har_FAR</i> from <i>Helicoverpa armigera</i>	<i>S. cerevisiae</i> CEN.PK1 02 -5B	pCfB4580 (pXI-KlLeu2syn-Har_FAR_HDEL_PTDH3 <->PTef1-Atf1)	1.4±0.4	1.9±0.1 %
ST5290	<i>ATF1</i> from <i>S. cerevisiae</i> , <i>Har_FAR</i> from <i>Helicoverpa armigera</i> , <i>DmeA9</i> from <i>D. melanogaster</i>	<i>S. cerevisiae</i> CEN.PK1 02 -5B	pCfB4580 (pXI-KlLeu2syn-Har_FAR_HDEL_PTDH3 <->PTef1-Atf1), pCfB531 6 (pXII-5-Dmd9-PTDH3<-)	7.3±0.2	9.8±0.4 %

Example 7: Method to produce Z 11-C14:0Ac

A gene, encoding a $\Delta 11$ desaturase that preferentially produces Z 11-C14:CoA is overexpressed in a yeast strain along with HarFAR and Atf1. The resulting strain is grown in cultivation medium and produces Z 11-14:OAc. The gene encodes for example the $\Delta 11$ desaturase from the oblique banded leaf roller moth *Choristoneura rosaceana* (SEQ ID NO: 65). The pheromone is recovered from the broth and formulated into mating disruption product to control pests, as e.g., European corn borer *Ostrinia nubilalis*.

Example 8: Method to produce E 11-C 14:0Ac

A gene, encoding a $\Delta 11$ desaturase that preferentially produces E 11-C14:CoA is overexpressed in a yeast strain along with HarFAR and Atf1. The resulting strain is grown in cultivation medium and produces E 11-14:OAc. The gene encodes for example the $\Delta 11$ desaturase from the spotted fireworm moth *Choristoneura parallela* (SEQ ID NO: 66). The pheromone is recovered from the broth and formulated into mating disruption product to control pests, as e.g., lightbrown apple moth *Epiphyas postvittana*.

Example 9: Construction of plasmids and Yarrowia lipolytica strains

Genes encoding desaturases from *Amyelois transitella* (SEQ ID NO: 68), *Spodoptera litura* (SEQ ID NO: 12) and *Drosophila melanogaster* (Dmd9; SEQ ID NO: 10), the fatty acyl reductase of *Helicoverpa armigera* (HarFAR; SEQ ID NO: 25), the thioesterases from *Escherichia coli* (SEQ ID NO: 42) and from *Cinnamomum camphora* (SEQ ID NO: 40) and the alcohol acetyltransferase of *Saccharomyces cerevisiae* (Atf1; SEQ ID NO: 21) were synthesized by GeneArt (Life Technologies) in codon-optimized versions for *Y. lipolytica*. The fatty acyl reductase of *Heliothis subflexa* was synthesized by GeneArt (Life technologies) in codon-optimized version for *Saccharomyces cerevisiae* (SEQ ID NO: 70).

In strain ST6629 the open-reading frame of genes *HFD4* (YALI0B01298g), *HFD3* (YALI0A17875), *HFD2* (YALI0E15400) and *HFD1* (YALI0F23793g), as well as nucleotides -1130 to -100 upstream of the coding sequence of *GPAT* (YALI0C00209g) were deleted. A premature Stop-codon and frame-shift was

introduced into *PEX10* (YALIOC01023g) and *FA01* (YALIOB14014g) resulting in non-functional genes.

Strain ST7394 is based on ST6629 and expresses Dmd9, HarFAR and Atf 1 as described in pCfB6969 and pCfB7600 (Fig. 2) from intergenic regions on chromosomes C (nucleotides 2192680-21 9371 0) and D (nucleotides 1842294-1 843343).

In strain ST6365, the open-reading frames of *HFD1*, *HFD4*, *PEX10*, and *FA01* were replaced with selection marker cassettes. ST6365 expressed the $\Delta 11$ desaturase of *A. transitella* and fatty acyl reductase from *Heliothis subflexa*.

Strain ST6357 expresses Atf 1 and HarFAR from an intergenic region on chromosome E (nucleotides 1722042-1 723055) as described in pCfB7235 (Fig. 2).

Strain ST6359 expresses Atf 1 and HarFAR from an intergenic region on chromosome E (nucleotides 1722042-1 723055) as described in pCfB7235 (Fig. 2) and Dmd9 from an intergenic region on chromosome E (2881519-2882566) as described in pCfB7239 (Fig. 2).

Strain ST6360 expresses Atf 1 and HarFAR from an intergenic region on chromosome E (nucleotides 1722042-1 723055) as described in pCfB7235 and SliDesl 1 from an intergenic region on chromosome E (2881519-2882566) as described in pCfB7240 (Fig. 2).

Strain ST6373 expresses Atf 1 and HarFAR from an intergenic region on chromosome E (nucleotides 1722042-1 723055) as described in pCfB7235 and Dmd9 and TesA(LL) from an intergenic region on chromosome E (2881 519-2882566) as described in pCfB7251 (Fig. 2).

Strain ST6375 expresses Atf 1 and HarFAR from an intergenic region on chromosome E (nucleotides 1722042-1 723055) as described in pCfB7235 and Dmd9 and CcFATBI from an intergenic region on chromosome E (2881 519-2882566) as described in pCfB7253 (Fig. 2).

In strain ST701 0 nucleotides 3658-3660 (ATC) of *Y. lipolytica*'s native fatty acyl synthetase 2 gene (YALI19382) were replaced by TTC.

5 In strain ST7895 and ST7944 the open-reading frames of genes *LIP2* and *LIP2 LIP8* were deleted, respectively.

Example 10: Method for increasing the production of (Z)9-14:OH and (Z)9-14:Ac in Yarrowia lipolytica by heterologous expression of thioesterases

10 The strains in table 9 were inoculated into 2 mL YPG medium (20 g/L peptone, 10 g/L yeast extract and 70 g/L glycerol) to an optical density (600 nm) of 1 and cultivated in 12-ml glass tubes (Duran, Wertheim, Germany) with metal labocap lids (Ludiswiss, Flawil, Switzerland) for 48 hours at 30 °C shaken at 250 rpm. If indicated the medium was supplemented with 1 g/L methyl myristate.

15 For fatty alcohol extraction, 1 mL of culture was transferred into a 4-mL glass vial and 10 μ L of internal standard solution (2 μ g/ μ L (Z)-10-heptan-1-yl methyl ester in 100% ethanol) was added. The vials were covered with small pieces of aluminum foil and a needle was used to pierce small holes in the foil covers. The samples were vortexed and placed at -80 °C for storage until analysis. The samples were freeze-dried in a freeze dry system (Freezone6 and Stoppering tray dryer, Labconco, Kansas City, USA) at -40 °C, then 1 mL chloroform :methanol 2:1 was added to disrupt the cells. The mix was vortexed for 45 s and left at room temperature for 4 hours. The organic solvents were evaporated slowly under a nitrogen stream. 1 mL of hexane was added, the samples were vortexed for 10 s, centrifuged and 200 μ L were transferred to a new glass vial. Quantification was performed with a SCION TQ GC-MS (Bruker), equipped with an INNOWax 30 m \times 0.25 mm \times 0.25 μ m column, with helium as carrier gas. The injector was configured in splitless mode at 250 °C, the oven temperature was set to 80 °C for 1 min, then increased at a rate of 10 °C /min to 210 °C , followed by a hold at 210 °C for 10 min, and then increased at a rate of 10 °C/min to 230 °C followed by a hold at 230 °C for 5 min. The MS was operated in electron impact mode (70eV), scanning 25 between m/z 30 and 350. Compounds were identified by comparison of retention times and mass spectra with those of reference compounds. Compounds were quantified by the Total Ion Current (TIC) recorded. Data were analyzed by the BrukerMSWorkstation software. The concentrations of fatty alcohols were calculated based on internal standards (Table 9).

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The example shows the production of (Z)9-14:OH and (Z)9-14:OAc in the yeast *V. lipolytica*. The additional expression of thioesterase either from *E. coli* or *C. camphora* increased the production of the compounds by 20% and 25%, respectively.

5 **Table 9. Increased production of (Z)9-14:OH and (Z)9-14:OAc in *Yarrowia lipolytica* by heterologous expression of thioesterases.** In the two right columns, the upper line indicates products in C14, the lower line products in C16. N.A.: not available.

Strain	Over-expressed genes	Parent strain	Plasmids integrated	Media supplementation	(Z)9-14:OH (Z)9-16:OH (mg/L)	(Z)9-14:0 Ac (Z)11-16:0 Ac (mg/L)
ST3683	<i>Yarrowia lipolytica</i> GB 20 (Angerer et al., 2014)			+ methyl myristate	0.1 ± 0.1	0.0 ± 0.0
ST6357	Har_FAR <i>ATF1</i>	ST6365	pCfB7235	+ methyl myristate	11.5 ± 1.5 1.8 ± 0.3	8.8 ± 0.6 33.6 ± 1.2
ST6359	Dmd9 Har_FAR <i>ATF1</i>	ST6365	pCfB7239 pCfB7235	+ methyl myristate	40.3 ± 7 1.2 ± 0.2	28 ± 1.0 22.6 ± 6.6
ST6360	Δ9 desaturase from <i>S. litura</i> Har_FAR <i>ATF1</i>	ST6365	pCfB7240 pCfB7235	+ methyl myristate	27.5 ± 0.9 3.6 ± 3.8	15.2 ± 0.8 98.6 ± 0.07
ST6373	Dmd9 Har_FAR <i>ATF1</i> Thioesterase from <i>E. coli</i>	ST6365	pCfB7251 pCfB7235	+ methyl myristate	83.3 ± 10.3 N.A.	50.7 ± 2.8 N.A.
ST6375	Dmd9	ST6365	pCfB7253	+ methyl	88.4 ± 5.8	50.6 ± 1.0

	Har_FAR <i>ATF1</i> Thioeste- rase from <i>C. cam- phora</i>		pCfB7235	myristate	N.A.	N.A.
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Example 11: Method for increasing production of (Z)9-14:OH in Yarrowia lipolytica by introducing point mutation in Yarrowia lipolytica fatty acyl synthetase (FAS2)

The strains in table 10 were cultivated as described in example 10, but the medium was not supplemented.

By introducing a point mutation (I1220F) in the native fatty acyl synthetase (FAS2) production of (Z)9-14:OH increased approximately 15 fold.

10 **Table 10**

Strain	Overexpressed genes	Parent strain	Plasmids integrated	(Z)9-14:OH (mg/L)	(Z)9-14:OAc (mg/L)
ST671 3	Δ 9 desaturase from <i>D. melanogaster</i> Fatty acyl reductase from <i>H. armigera</i>	ST6629	pCfB6969	4.9 \pm 1.4	-
ST701 0	Δ 9 desaturase from <i>D. melanogaster</i> Fatty acyl reductase from <i>H. armigera</i> YLFAS2 (I1 220F)	ST6629	pCfB6969	73.6 \pm 16.2	-

Example 12: Method for increasing production of (Z)9- 14:Ac in Yarrowia lipolytica by deletion of Yarrowia lipolytica lipase genes

The stains in table 11 were cultivated as described in example 10. The medium was supplemented with 1 g/L methyl myristate. Deletion of lipase 2 alone or lipase 2 and lipase 8 together resulted in increased fatty alcohol titers, as can be seen in fig. 3.

Table 11

Strain	Overexpressed genes	Parent strain	Plasmids integrated
ST7394	$\Delta 9$ desaturase from <i>D. melanogaster</i> Fatty acyl reductase from <i>H. armigera</i> Alcohol acetyltransferase from <i>S. cerevisiae</i>	ST6629	pCfB6969 pCfB7600
ST7895	$\Delta 9$ desaturase from <i>D. melanogaster</i> Fatty acyl reductase from <i>H. armigera</i> Alcohol acetyltransferase from <i>S. cerevisiae</i> $\Delta lip2$	ST6629	pCfB6969 pCfB7600
ST7944	$\Delta 9$ desaturase from <i>D. melanogaster</i> Fatty acyl reductase from <i>H. armigera</i> Alcohol acetyltransferase from <i>S. cerevisiae</i> $\Delta lip2 \Delta lip8$	ST6629	pCfB6969 pCfB7600

Sequences

10 Overview

SEQ ID NO: 1 - *Y. lipolytica* codon-optimized nucleotide sequence of $\Delta 9$ desaturase from *Pelargonium hortorum*

SEQ ID NO: 2 - $\Delta 9$ desaturase from *Pelargonium hortorum*

15 SEQ ID NO: 3 - *Y. lipolytica* codon-optimized nucleotide sequence of $\Delta 9$ desaturase from *Ricinus communis*

SEQ ID NO: 4 - $\Delta 9$ desaturase from *Ricinus communis*

SEQ ID NO: 5 - *S. cerevisiae* codon-optimized nucleotide sequence of $\Delta 9$ desaturase from *Amyelopsis transitella* Atr236

SEQ ID NO: 6 - $\Delta 9$ desaturase from *Amyelopsis transitella* Atr236

20 SEQ ID NO: 7 - *S. cerevisiae* codon-optimized nucleotide sequence of $\Delta 9$ desaturase from *Amyelopsis transitella* Atr1432

- SEQ ID NO: 8 - $\Delta 9$ desaturase from *Amyelois transitella* Atr1432
- SEQ ID NO: 9 - *S. cerevisiae* codon-optimized nucleotide sequence of $\Delta 9$ desaturase from *Drosophila melanogaster* Dmd9
- SEQ ID NO: 10 - $\Delta 9$ desaturase from *Drosophila melanogaster* Dmd9
- 5 SEQ ID NO: 11 - *Y. lipolytica* codon-optimized nucleotide sequence of $\Delta 9$ desaturase from *Spodoptera litura* Des1 1
- SEQ ID NO: 12 - $\Delta 9$ desaturase from *Spodoptera litura* Des1 1
- SEQ ID NO: 13 - *V. lipolytica* codon-optimized nucleotide sequence of $\Delta 9$ desaturase from *Chauliognathus lugubris* Cld9
- 10 SEQ ID NO: 14 - $\Delta 9$ desaturase from *Chauliognathus lugubris* Cld9
- SEQ ID NO: 15 - *V. lipolytica* codon-optimized nucleotide sequence of desaturase from *Tribolium castaneum* D6
- SEQ ID NO: 16 - desaturase from *Tribolium castaneum* D6
- SEQ ID NO: 17 - *Y. lipolytica* codon-optimized nucleotide sequence of desaturase from
- 15 *Tribolium castaneum* D8
- SEQ ID NO: 18 - desaturase from *Tribolium castaneum* D8
- SEQ ID NO: 19 - *Saccharomyces cerevisiae* ATF1 DNA sequence; DNA coding sequence.
- SEQ ID NO: 20 - *Y. lipolytica* codon-optimized nucleotide sequence of alcohol
- 20 acetyltransferase from *S. cerevisiae* ATF1
- SEQ ID NO: 21 - *Saccharomyces cerevisiae* ATF1p amino acid sequence
- SEQ ID NO: 22 - *Y. lipolytica* codon-optimized nucleotide sequence of thioesterase from *Cuphea palustris* CpFATB2
- SEQ ID NO: 23 - protein sequence of thioesterase from *Cuphea palustris* CpFATB2
- 25 SEQ ID NO: 24 - *S. cerevisiae*-codon-optimized nucleotide sequence of *Helicoverpa armigera* fatty acyl reductase; mRNA-coding sequence
- SEQ ID NO: 25 - *H. armigera* fatty acyl reductase
- SEQ ID NO: 26 - *S. cerevisiae*-codon-optimized nucleotide sequence of *H. armigera* fatty acyl reductase with signal peptide changed to HDEL; DNA coding sequence.
- 30 SEQ ID NO: 27 - *H. armigera* fatty acyl reductase with signal peptide changed to HDEL
- SEQ ID NO: 28 - *S. cerevisiae*-codon-optimized nucleotide sequence of *H. assulta* fatty acyl reductase; mRNA-coding sequence.
- SEQ ID NO: 29 - Amino acid sequence of *H. assulta* fatty acyl reductase
- SEQ ID NO: 30 - *S. cerevisiae*-codon-optimized nucleotide sequence of *Helicoverpa*
- 35 *assulta* fatty acyl reductase with signal peptide changed to HDEL; mRNA-coding sequence

- SEQ ID NO: 31 - amino acid sequence of *H. assulta* fatty acyl reductase with signal peptide changed to HDEL
- SEQ ID NO: 32 - S. cerevisiae-codon-optimized nucleotide sequence of *Heliothis subflexa* fatty acyl reductase; mRNA-coding sequence.
- 5 SEQ ID NO: 33 - Amino acid sequence of *H. subflexa* fatty acyl reductase
- SEQ ID NO: 34 - S. cerevisiae-codon-optimized nucleotide sequence of *H. subflexa* fatty acyl reductase with signal peptide changed to HDEL; mRNA-coding sequence
- SEQ ID NO: 35 - amino acid sequence of *H. subflexa* fatty acyl reductase with signal peptide changed to HDEL
- 10 SEQ ID NO: 36 - *Y. lipolytica* codon-optimized nucleotide sequence of $\Delta 9$ desaturase from *Drosophila melanogaster* Dmd9
- SEQ ID NO: 37 - *Y. lipolytica* codon-optimised nucleotide sequence of thioesterase from *Cuphea hookeriana* ChFatB3
- SEQ ID NO: 38 - amino acid sequence of thioesterase from *Cuphea hookeriana*
- 15 ChFatB3
- SEQ ID NO: 39 - *Y. lipolytica* codon-optimised nucleotide sequence of thioesterase from *Cinnamomum camphora* CcFatBI
- SEQ ID NO: 40 - amino acid sequence of thioesterase from *Cinnamomum camphora* CcFatBI
- 20 SEQ ID NO: 41 - *Y. lipolytica* codon-optimized nucleotide sequence of thioesterase from *Escherichia coli* TesA, without the leader sequence, named TesA(LL)
- SEQ ID NO: 42 - protein sequence of thioesterase from *Escherichia coli* TesA, without the leader sequence, named TesA(LL)
- SEQ ID NO: 43 - *Y. lipolytica* codon-optimized nucleotide sequence of fatty acyl
- 25 reductase from *H. armigera* Har_FAR
- SEQ ID NO: 44 - *Y. lipolytica* codon-optimized nucleotide sequence of fatty acyl reductase from *Bicyclus anynana* Ban-wFAR2
- SEQ ID NO: 45 - protein sequence of fatty acyl reductase from *Bicyclus anynana* Ban-wFAR2
- 30 SEQ ID NO: 46 - PR-1852 (PTDH3_fw)
- SEQ ID NO: 47 PR-1853 (PTDH3_rv)
- SEQ ID NO: 48 PR-1565 (PTEF1)
- SEQ ID NO: 49 PR-8332 (Har_FAR_U1_fw)
- SEQ ID NO: 50 PR-10739 (Har_FAR_HDEL_U1_rev)
- 35 SEQ ID NO: 51 PR-14318 (Phd9_U2_fw)
- SEQ ID NO: 52 PR-14276 (Phd9_U2_rev)

- SEQ ID NO: 53 PR-14319 (RCd9_U2_fw)
 SEQ ID NO: 54 PR-14278 (RCd9_U2_rev)
 SEQ ID NO: 55 PR-14320 (Atf1_U2_fw)
 SEQ ID NO: 56 PR-14321 (Atf1_U2_rev)
 5 SEQ ID NO: 57 PR-15974 (Dmd9_U1_fw)
 SEQ ID NO: 58 PR-15975 (Dmd9_U1_rev)
 SEQ ID NO: 59 PR-15976 (attB1_Dmd9_F)
 SEQ ID NO: 60 PR-15977 (attB2_Dmd9_R)
 SEQ ID NO: 61 PR-15978 (attB1_Ph9_F)
 10 SEQ ID NO: 62 PR-15979 (attB2_Ph9_R)
 SEQ ID NO: 63 PR-15980 (attB1_Rcd9_F)
 SEQ ID NO: 64 PR-15981 (attB1_Rcd9_R)
 SEQ ID NO: 65 Δ 11 desaturase from *Choristoneura rosaceana*.
 SEQ ID NO: 66 - Δ 11 desaturase from *Choristoneura parallela*
 15 SEQ ID NO: 67 - *Y. lipolytica* codon-optimized nucleotide sequence of *Amyelois transitella* Δ 11 desaturase
 SEQ ID NO: 68 - Δ 11 desaturase from *Amyelois transitella*
 SEQ ID NO: 69 - *Y. lipolytica* codon-optimized nucleotide sequence of *Helicoverpa armigera* fatty acyl reductase
 20 SEQ ID NO: 70 - *S. cerevisiae* codon-optimized nucleotide sequence of *Heliothis subflexa* fatty acyl reductase
 SEQ ID NO: 71: FAS2 sequence (wild type)
 SEQ ID NO: 72: Sequence of LIP2 from *Yarrowia lipolytica*.
 SEQ ID NO: 73: Sequence of LIP7 from *Y. lipolytica*
 25 SEQ ID NO: 74: Sequence of LIP8 from *Y. lipolytica*

References

- Alfaro, Navarro-Llopis, Primo, 2009. Optimization of pheromone dispenser density for managing the rice striped stem borer, *Chilo suppressalis* (Walker), by mating disruption. Crop Protection. 28:567-572.
 30 Bari, 2003. Development of pheromone mating disruption strategies for the suppression of the artichoke plume moth in artichokes grown on the central coast of California. ISHS Acta Horticulturae 660: V International Congress on Artichoke. doi: 10.17660/ActaHortic.2004.660.80
 Eizaguirre, Sans, Lopez, Albajes. 2002. Effects of mating disruption against the
 35 Mediterranean corn borer, *Sesamia nonagrioides*, on the European corn borer *Ostrinia*

- nubilalis. Use of pheromones and other semiochemicals in integrated production IOBC wprs Bulletin.
- Ferrell, Yao, 1972. Reductive and oxidative synthesis of saturated and unsaturated fatty aldehydes, *J Lipid Res.* 13(1):23-6.)-
- 5 Gietz RD, Schiestl RH. High-efficiency yeast transformation using the LiAc/SS carrier DNA/PEG method. *Nat Protoc.* 2007;2(1):31-4.
- Jessop-Fabre MM, Jakociunas T, Stovicek V, Dai Z, Jensen MK, Keasling JD, Borodina I. EasyClone-MarkerFree: A vector toolkit for marker-less integration of genes into *Saccharomyces cerevisiae* via CRISPR-Cas9. *Biotechnol J.* 2016
- 10 Aug;11(8):1110-7.
- Jensen, Strucko, Kildegaard, David, Maury, Mortensen, Forster, Nielsen, Borodina, 2014. EasyClone: method for iterative chromosomal integration of multiple genes in *Saccharomyces cerevisiae*, *FEMS Yeast Res.* 14(2):238-48
- Kehat, Dunkelblum, 1993. Sex Pheromones: achievements in monitoring and mating disruption of cotton pests in Israel, *Achievements of Insect Biochemistry and Physiology.* 15 22:425-431 .
- Li, Zhang, 2009. An environmentally benign TEMPO-catalyzed efficient alcohol oxidation system with a recyclable hypervalent iodine(III) reagent and its facile preparation. *Synthesis*, 1163-1169a.
- 20 Meyer, Schreiber, 1994. Acceleration of the Dess-Martin oxidation by water *J. Org. Chem.*, 59, 7549-7552;
- Mitchell & McLaughlin (1982), "Suppression of Mating and Oviposition by Fall Armyworm and Mating by Corn Earworm in Corn, Using the Air Permeation Technique", *Journal of Economic Entomology*, 75(2):270-274
- 25 Okada, Asawa, Sugiyama, Kirihaara, Iwai, Kimura, 2014. Sodium hypochlorite pentahydrate (NaOCl·5H₂O) crystals as an extraordinary oxidant for primary and secondary alcohols. *Synlett*, 25, 596-598.
- Schneider, R., Tatzer, V., Gogg, G., Leitner, E., Kohlwein, S.D., 2000. Elongation of C₁₄:Δ⁹ to C₁₆:Δ¹¹ fatty acids in *Saccharomyces*
- 30 *cerevisiae*. *J. Bacteriol.* 182, 3655-3660.
- Steves J.E. and Stahl S.S., 2013. Copper(I)/ABNO-catalyzed aerobic alcohol oxidation: alleviating steric and electronic constraints of Cu/TEMPO catalyst systems. *J. Am. Chem. Soc.* 135, 15742-15745
- Stovicek V, Borja GM, Forster J, Borodina I. EasyClone 2.0: expanded toolkit of
- 35 integrative vectors for stable gene expression in industrial *Saccharomyces cerevisiae* strains. *J Ind Microbiol Biotechnol.* 2015 Nov;42(11):1519-31 .

Tamura, Aoyama, Takido, Kodomari, 2012. Novel [4-Hydroxy-TEMPO + NaCl]/SiO₂ as a reusable catalyst for aerobic oxidation of alcohols to carbonyls. *Synlett*, 23, 1397-1407.

5 Wu, Zhang, Yao, Xu, Wang and Zhang, 2012. Management of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) by mating disruption. *Insect Science* 19 (6), 643-648.

Yadav, Reddy, Basak, Narsaiah, 2004. Recyclable 2nd generation ionic liquids as green solvents for the oxidation of alcohols with hypervalent iodine reagents, *Tetrahedron*, 60, 2131-2135.

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Items

1. A yeast cell capable of producing a desaturated fatty alcohol and optionally a desaturated fatty acyl acetate, said yeast cell expressing:
 - 15 i) at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA having a carbon chain length of 14; and
 - ii) at least one heterologous fatty acyl-CoA reductase (FAR), capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol; and
 - 20 iii) optionally an acetyltransferase capable of converting at least part of said desaturated fatty alcohol to a desaturated fatty acyl acetate;

wherein the desaturase has a higher specificity towards tetradecanoyl-CoA than towards hexadecanoyl-CoA and/or wherein the fatty acyl-CoA reductase has a higher specificity towards desaturated tetradecanoyl-CoA than towards desaturated hexadecanoyl-CoA.

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2. The yeast cell according to item 1, wherein the at least one heterologous desaturase is selected from the group consisting of a Δ^3 desaturase, a Δ^5 desaturase, a Δ^6 desaturase, a Δ^7 desaturase, a Δ^8 desaturase, a Δ^9 desaturase, a Δ^{10} desaturase, a Δ^{11} desaturase, a Δ^{12} desaturase and a Δ^{13} desaturase.

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3. The yeast cell according to any one of the preceding items, wherein the desaturase is capable of introducing at least one double bond in position 5, 6, 7, 8, 9, 10, 11, 12 or 13.

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4. The yeast cell according to any one of the preceding items, wherein the desaturase is derived from an organism selected from *Pelargonium hortorum*, *Ricinus communis*, *Drosophila melanogaster*, *Spodoptera litura* and *Tribolium castaneum*, preferably the desaturase is derived from *Drosophila melanogaster*.

5

5. The yeast cell according to any one of the preceding items, wherein the at least one heterologous desaturase is selected from the group consisting of:

- i) a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Pelargonium hortorum* as set forth in SEQ ID NO: 2;
 - 10 ii) a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Ricinus communis* as set forth in SEQ ID NO: 4;
 - iii) a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 10;
 - 15 iv) a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Spodoptera litura* as set forth in SEQ ID NO: 12;
 - v) a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Chauliognathus lugubris* as set forth in SEQ ID NO: 14;
 - vi) a desaturase having at least 60% homology to the desaturase from *Tribolium castaneum* as set forth in SEQ ID NO: 16; and
 - 20 vii) a desaturase having at least 60% homology to the desaturase from *Tribolium castaneum* as set forth in SEQ ID NO: 18;
 - viii) a $\Delta 11$ desaturase having at least 60% homology to the desaturase from *Choristoneura rosaceana* as set forth in SEQ ID NO: 65;
 - 25 ix) a $\Delta 11$ desaturase having at least 60% homology to the desaturase from *Choristoneura parallela* as set forth in SEQ ID NO: 66,
- preferably the desaturase is a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 10 or a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Spodoptera litura* as set forth in SEQ ID NO: 12.

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6. The yeast cell according to any one of the preceding items, wherein the at least one heterologous desaturase is selected from the group consisting of:

- i) a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 10;
- 35 ii) a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Spodoptera litura* as set forth in SEQ ID NO: 12;

- iii) a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Chauliognathus lugubris* as set forth in SEQ ID NO: 14;
- iv) a desaturase having at least 60% homology to the desaturase from *Tribolium castaneum* as set forth in SEQ ID NO: 16; and
- 5 v) a desaturase having at least 60% homology to the desaturase from *Tribolium castaneum* as set forth in SEQ ID NO: 18;
- vi) a $\Delta 11$ desaturase having at least 60% homology to the desaturase from *Choristoneura rosaceana* as set forth in SEQ ID NO: 65;
- vii) a $\Delta 11$ desaturase having at least 60% homology to the desaturase from *Choristoneura parallela* as set forth in SEQ ID NO: 66.
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7. The yeast cell according to any one of the preceding items, wherein the fatty acyl-CoA reductase (FAR) is selected from:
- i) a FAR having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 25 or SEQ ID NO: 27;
- 15 ii) a FAR having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 29 or SEQ ID NO: 31;
- iii) a FAR having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 33 or SEQ ID NO: 35; and
- 20 iv) a FAR having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 45,
- preferably the FAR is a FAR having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 25 or SEQ ID NO: 27.
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8. The yeast cell according to any one of the preceding items, wherein the acetyltransferase is a heterologous acetyltransferase expressed from said yeast cell or a native acetyltransferase overexpressed from said yeast cell.
9. The yeast cell according to any one of the preceding items, wherein the acetyltransferase has at least 75% homology to the acetyltransferase Atf 1 from *Saccharomyces cerevisiae* as set forth in SEQ ID NO: 21.
- 30
10. The yeast cell according to any one of the preceding items, wherein the yeast is of a genus selected from *Saccharomyces*, *Pichia*, *Yarrowia*, *Kluyveromyces*, *Candida*, *Rhodotorula*, *Rhodospiridium*, *Cryptococcus*, *Trichosporon* and
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Lipomyces, preferably the genus is *Saccharomyces* or *Yarrowia*, most preferably the genus is *Yarrowia*.

5 11. The yeast cell according to any one of the preceding items, wherein the yeast is of a species selected from *Saccharomyces cerevisiae*, *Pichia pastoris*, *Kluyveromyces marxianus*, *Cryptococcus albidus*, *Lipomyces lipofera*, *Lipomyces starkeyi*, *Rhodospiridium toruloides*, *Rhodotorula glutinis*, *Trichosporon pullulan* and *Yarrowia lipolytica*, preferably the yeast cell is a *Saccharomyces cerevisiae* cell or a *Yarrowia lipolytica* cell, most preferably the yeast cell is a *Yarrowia lipolytica* cell.

12. The yeast cell according to any one of the preceding items, wherein the cell:

15 i) expresses a $\Delta 9$ desaturase identical to or having at least 60% homology to the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 10; and

ii) expresses a fatty acyl-CoA reductase identical to or having at least 80% homology to the fatty acyl-CoA reductase from *Helicoverpa armigera* as set forth in SEQ ID NO: 25; and

20 iii) expresses or overexpresses an acetyltransferase identical to or having at least 75% homology to the acetyltransferase from *Saccharomyces cerevisiae* as set forth in SEQ ID NO: 21.

13. The yeast cell according to any one of the preceding items, wherein the acetyltransferase is overexpressed compared to a wild type yeast cell.

25 14. The yeast cell of any one of the preceding items, wherein the genes encoding the desaturase, the fatty acyl-CoA reductase, or the acetyltransferase are comprised within the genome of said yeast cell or within one or more vector comprised within said yeast cell.

30 15. The yeast cell of any one of the preceding items, wherein the yeast cell further expresses or overexpresses a thioesterase.

35 16. The yeast cell of any one of the preceding items, wherein the thioesterase has at least 60% homology to the thioesterase from *Cuphea palustris* as set forth in SEQ ID NO: 23, to the thioesterase from *Cuphea hookeriana* as set forth in

5 SEQ ID NO: 38, to the thioesterase from *Cinnamomum camphora* as set forth in SEQ ID NO: 40, or to the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 42, preferably the thioesterase has at least 60% homology to the thioesterase from *Cinnamomum camphora* as set forth in SEQ ID NO: 40, or to the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 42.

10 17. The yeast cell of any one of the preceding items, wherein the yeast cell further expresses a fatty acyl synthase variant having a modified ketone synthase domain, whereby the variant preferably binds shorter fatty acids.

18. The yeast cell of any one of the preceding items, said yeast cell further having a mutation resulting in partial or total loss of activity of one or more lipases.

15 19. The yeast cell according to item 18, wherein the one or more lipases has at least 60% homology to lipase 2 of *Yarrowia lipolytica* as set forth in SEQ ID NO: 72, lipase 7 of *Yarrowia lipolytica* as set forth in SEQ ID NO: 73, or lipase 8 of *Yarrowia lipolytica* as set forth in SEQ ID NO: 74.

20 20. The yeast cell according to any one of items 18 to 19, wherein the yeast cell is *Yarrowia lipolytica* and the one or more lipases are selected from the group consisting of lipase 2 as set forth in SEQ ID NO: 72, lipase 7 as set forth in SEQ ID NO: 73 and lipase 8 as set forth in SEQ ID NO: 74.

25 21. The yeast cell of any one of the preceding items, wherein at least one of the genes encoding the desaturase, the fatty acyl-CoA reductase, the acetyltransferase or the thioesterase is present in high copy number.

30 22. The yeast cell of any one of the preceding items, wherein at least one of the genes encoding the desaturase, the fatty acyl-CoA reductase, the acetyltransferase or the thioesterase is under the control of an inducible promoter.

35 23. The yeast cell of any one of the preceding items, wherein at least one of the genes encoding the desaturase, the fatty acyl-CoA reductase, the acetyltransferase or the thioesterase is codon-optimised for said yeast cell.

24. A method for production of a desaturated fatty acid and optionally a desaturated fatty acyl acetate in a yeast cell, said method comprising the steps of providing a yeast cell and incubating said yeast cell in a medium, wherein the yeast cell expresses:

- i) at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA having a carbon chain length of 14, thereby converting at least part of said fatty acyl-CoA to a desaturated fatty acyl-CoA; and
- ii) at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, thereby producing said desaturated fatty alcohol; and
- iii) optionally an acetyltransferase capable of converting at least part of said desaturated fatty alcohol to a desaturated fatty acyl acetate, thereby producing said desaturated fatty acyl acetate;

wherein the desaturase has a higher specificity towards tetradecanoyl-CoA than towards hexadecanoyl-CoA and/or wherein the fatty acyl-CoA reductase has a higher specificity towards desaturated tetradecanoyl-CoA than towards desaturated hexadecanoyl-CoA.

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25. The method according to item 24, wherein the yeast cell is as defined in any one of items 1 to 23.

26. The method according to any one of items 24 to 25, wherein the ratio of desaturated tetradecanoyl-CoA to desaturated hexadecanoyl-CoA is of at least 0.1, such as at least 0.2, such as at least 0.3, such as at least 0.4, such as at least 0.5, such as at least 0.75, such as at least 1, such as at least 2, such as at least 3, such as at least 4, such as at least 5, such as at least 6, such as at least 7, such as at least 8, such as at least 9, such as at least 10, such as at least 12.5, such as at least 15.

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27. The method according to any one of items 25 to 26, wherein the method yields desaturated fatty alcohols with a titre of at least 1 mg/L, such as at least 1.5 mg/L, such as at least 5 mg/L, such as at least 10 mg/L, such as at least 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 750 mg/L, such as at least 1

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g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, or more.

- 5 28. The method according to any one of items 25 to 27, wherein the method yields a desaturated fatty alcohol having a chain length of 14 with a titre of at least 1 mg/L, such as at least 1.5 mg/L, such as at least 5 mg/L, such as at least 10 mg/L, such as at least 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 750 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, 10 such as at least 4 g/L, such as at least 5 g/L, or more.
- 15 29. The method according to any one of items 25 to 28, wherein the method yields desaturated fatty acyl acetates with a titre of at least 1 mg/L, such as at least 1.5 mg/L, such as at least 5 mg/L, such as at least 10 mg/L, such as at least 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 750 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, or more.
- 20 30. The method according to any one of items 25 to 29, wherein the method yields a desaturated fatty acyl acetate having a chain length of 14 with a titre of at least 1 mg/L, such as at least 1.5 mg/L, such as at least 5 mg/L, such as at least 10 mg/L, such as at least 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 750 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, or more. 25
- 30 31. The method according to any one of items 25 to 30, wherein the desaturated fatty acyl acetates comprise at least 1% of a desaturated fatty acyl acetate having a chain length of 14, such as at least 1.5%, such as at least 2%, such as at least 2.5%, such as at least 3%, such as at least 3.5%, such as at least 4%, such as at least 4.5%, such as at least 5%, such as at least 7.5%, such as at least 10%.
- 35 32. The method according to any one of items 25 to 31, wherein the yeast cell is further capable of expressing a thioesterase.

33. The method according to any one of items 25 to 32, wherein the thioesterase has at least 60% homology to the thioesterase from *Cuphea palustris* as set forth in SEQ ID NO: 23, to the thioesterase from *Cuphea hookeriana* as set forth in SEQ ID NO: 38, to the thioesterase from *Cinnamomum camphora* as set forth in SEQ ID NO: 40, or to the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 42.
34. The method according to any one of items 25 to 33, further comprising the step of recovering said desaturated fatty alcohol and/or desaturated fatty acyl acetate.
35. The method according to any one of items 25 to 34, further comprising the step of formulating the recovered desaturated fatty alcohol and/or desaturated fatty acyl acetate into a pheromone composition.
36. The method according to any one of items 25 to 35, wherein the pheromone composition further comprises one or more additional compounds such as a liquid or solid carrier or substrate.
37. A nucleic acid construct for modifying a yeast cell, said construct comprising:
- i) a first polynucleotide encoding at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA having a carbon chain length of 14; and
 - ii) a second polynucleotide encoding at least one heterologous fatty acyl-CoA reductase (FAR), capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol; and
 - iii) optionally a third polynucleotide encoding an acetyltransferase capable of converting at least part of said desaturated fatty alcohol to a desaturated fatty acyl acetate,
- wherein optionally the first polynucleotide, the second polynucleotide and/or the third polynucleotide are under the control of a promoter.
38. A kit of parts comprising:
- a) the yeast cell according to any one of items 1 to 24 and instructions for use; and/ or

- b) a nucleic acid construct according to item 37, wherein said construct is for modifying a yeast cell, and
- c) optionally the yeast cell to be modified.

5 39. A desaturated fatty alcohol obtainable by the method according to any one of items 25 to 36.

40. A desaturated fatty acyl acetate obtainable by the method according to any one of items 35 to 36.

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41. Use of a desaturated fatty alcohol according to any one of items 1 to 24 or 39.

42. Use of a desaturated fatty fatty acyl acetate according to any one of items 1 to 24 or 40.

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Claims

1. A yeast cell capable of producing a desaturated fatty alcohol and optionally a desaturated fatty acyl acetate, said yeast cell expressing:
- 5 i) at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA having a carbon chain length of 14; and
- ii) at least one heterologous fatty acyl-CoA reductase (FAR), capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol; and
- 10 iii) optionally an acetyltransferase capable of converting at least part of said desaturated fatty alcohol to a desaturated fatty acyl acetate;
- wherein the desaturase has a higher specificity towards tetradecanoyl-CoA than towards hexadecanoyl-CoA and/or wherein the fatty acyl-CoA reductase has a higher specificity towards desaturated tetradecanoyl-CoA than towards desaturated hexadecanoyl-CoA.
- 15
2. The yeast cell according to any one of the preceding claims, wherein the desaturase is derived from an organism selected from *Pelargonium hortorum*, *Ricinus communis*, *Drosophila melanogaster*, *Spodoptera litura* and *Tribolium castaneum*, preferably the desaturase is derived from *Drosophila melanogaster*.
- 20
3. The yeast cell according to any one of the preceding claims, wherein the at least one heterologous desaturase is selected from the group consisting of:
- i) a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Drosophila melanogaster* as set forth in SEQ ID NO: 10;
- 25 ii) a $\Delta 9$ desaturase having at least 60% homology to the $\Delta 9$ desaturase from *Spodoptera litura* as set forth in SEQ ID NO: 12.
4. The yeast cell according to any one of the preceding claims, wherein the fatty acyl-CoA reductase (FAR) is selected from:
- 30 i) a FAR having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 25 or SEQ ID NO: 27;
- ii) a FAR having at least 80% homology to the FAR from *Helicoverpa assulta* as set forth in SEQ ID NO: 29 or SEQ ID NO: 31;
- iii) a FAR having at least 80% homology to the FAR from *Heliothis subflexa* as set forth in SEQ ID NO: 33 or SEQ ID NO: 35; and
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- iv) a FAR having at least 80% homology to the FAR from *Bicyclus anynana* as set forth in SEQ ID NO: 45,
preferably the FAR is a FAR having at least 80% homology to the FAR from *Helicoverpa armigera* as set forth in SEQ ID NO: 25 or SEQ ID NO: 27.

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5. The yeast cell according to any one of the preceding claims, wherein the acetyltransferase is a heterologous acetyltransferase expressed from said yeast cell or a native acetyltransferase overexpressed from said yeast cell, preferably the acetyltransferase has at least 75% homology to the acetyltransferase Atf1 from *Saccharomyces cerevisiae* as set forth in SEQ ID NO: 21.
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6. The yeast cell according to any one of the preceding claims, wherein the yeast is of a genus selected from *Saccharomyces*, *Pichia*, *Yarrowia*, *Kluyveromyces*, *Candida*, *Rhodotorula*, *Rhodospiridium*, *Cryptococcus*, *Trichosporon* and *Lipomyces*, preferably the genus is *Saccharomyces* or *Yarrowia*, most preferably the genus is *Saccharomyces* or *Yarrowia*, preferably the yeast is of a species selected from *Saccharomyces cerevisiae*, *Pichia pastoris*, *Kluyveromyces marxianus*, *Cryptococcus albidus*, *Lipomyces lipoferula*, *Lipomyces starkeyi*, *Rhodospiridium toruloides*, *Rhodotorula glutinis*, *Trichosporon pullulan* and *Yarrowia lipolytica*, preferably the yeast cell is a *Saccharomyces cerevisiae* cell or a *Yarrowia lipolytica* cell, most preferably the yeast cell is a *Yarrowia lipolytica* cell.
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7. The yeast cell of any one of the preceding claims, wherein the yeast cell further expresses or overexpresses a thioesterase, preferably the thioesterase has at least 60% homology to the thioesterase from *Cinnamomum camphora* as set forth in SEQ ID NO: 40, or to the thioesterase from *Escherichia coli* as set forth in SEQ ID NO: 42.
- 25
8. The yeast cell of any one of the preceding claims, wherein the yeast cell further expresses a fatty acyl synthase variant having a modified ketone synthase domain, whereby the yeast cell synthesises a higher proportion of C 14 fatty acids than a yeast cell expressing a native fatty acyl synthase in the same conditions.
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9. The yeast cell of any one of the preceding claims, said yeast cell further having a mutation resulting in partial or total loss of activity of one or more lipases.
10. The yeast cell according to claim 9, wherein the one or more lipases has at least 60% homology to lipase 2 of *Yarrowia lipolytica* as set forth in SEQ ID NO: 72, lipase 7 of *Yarrowia lipolytica* as set forth in SEQ ID NO: 73, or lipase 8 of *Yarrowia lipolytica* as set forth in SEQ ID NO: 74.
11. The yeast cell according to any one of claims 9 to 10, wherein the yeast cell is *Yarrowia lipolytica* and the one or more lipases are selected from the group consisting of lipase 2 as set forth in SEQ ID NO: 72, lipase 7 as set forth in SEQ ID NO: 73 and lipase 8 as set forth in SEQ ID NO: 74.
12. A method for production of a desaturated fatty acid and optionally a desaturated fatty acyl acetate in a yeast cell, said method comprising the steps of providing a yeast cell and incubating said yeast cell in a medium, wherein the yeast cell expresses:
- i) at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA having a carbon chain length of 14, thereby converting at least part of said fatty acyl-CoA to a desaturated fatty acyl-CoA; and
 - ii) at least one heterologous fatty acyl-CoA reductase, capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol, thereby producing said desaturated fatty alcohol; and
 - iii) optionally an acetyltransferase capable of converting at least part of said desaturated fatty alcohol to a desaturated fatty acyl acetate, thereby producing said desaturated fatty acyl acetate;
- wherein the desaturase has a higher specificity towards tetradecanoyl-CoA than towards hexadecanoyl-CoA and/or wherein the fatty acyl-CoA reductase has a higher specificity towards desaturated tetradecanoyl-CoA than towards desaturated hexadecanoyl-CoA.
13. The method according to claim 12, wherein the yeast cell is as defined in any one of claims 1 to 7.

14. The method according to any one of claims 12 to 13, wherein the method yields a desaturated fatty alcohol having a chain length of 14 with a titre of at least 1 mg/L, such as at least 1.5 mg/L, such as at least 5 mg/L, such as at least 10 mg/L, such as at least 25 mg/L, such as at least 50 mg/L, such as at least 100 mg/L, such as at least 250 mg/L, such as at least 500 mg/L, such as at least 750 mg/L, such as at least 1 g/L, such as at least 2 g/L, such as at least 3 g/L, such as at least 4 g/L, such as at least 5 g/L, or more.

15. A nucleic acid construct for modifying a yeast cell, said construct comprising:

- i) a first polynucleotide encoding at least one heterologous desaturase capable of introducing at least one double bond in a fatty acyl-CoA having a carbon chain length of 14; and
- ii) a second polynucleotide encoding at least one heterologous fatty acyl-CoA reductase (FAR), capable of converting at least part of said desaturated fatty acyl-CoA to a desaturated fatty alcohol; and
- iii) optionally a third polynucleotide encoding an acetyltransferase capable of converting at least part of said desaturated fatty alcohol to a desaturated fatty acyl acetate,

wherein optionally the first polynucleotide, the second polynucleotide and/or the third polynucleotide are under the control of a promoter.

16. A kit of parts comprising:

- a) the yeast cell according to any one of claims 1 to 11 and instructions for use; and/ or
- b) a nucleic acid construct according to claim 15, wherein said construct is for modifying a yeast cell, and
- c) optionally the yeast cell to be modified.

17. A desaturated fatty alcohol or a desaturated fatty acyl acetate obtainable by the method according to any one of claims 12 to 14.

18. Use of a desaturated fatty alcohol according to any one of claims 1 to 11 or 17.

19. Use of a desaturated fatty acyl acetate according to any one of claims 1 to 7 or 17.

FIG. 1

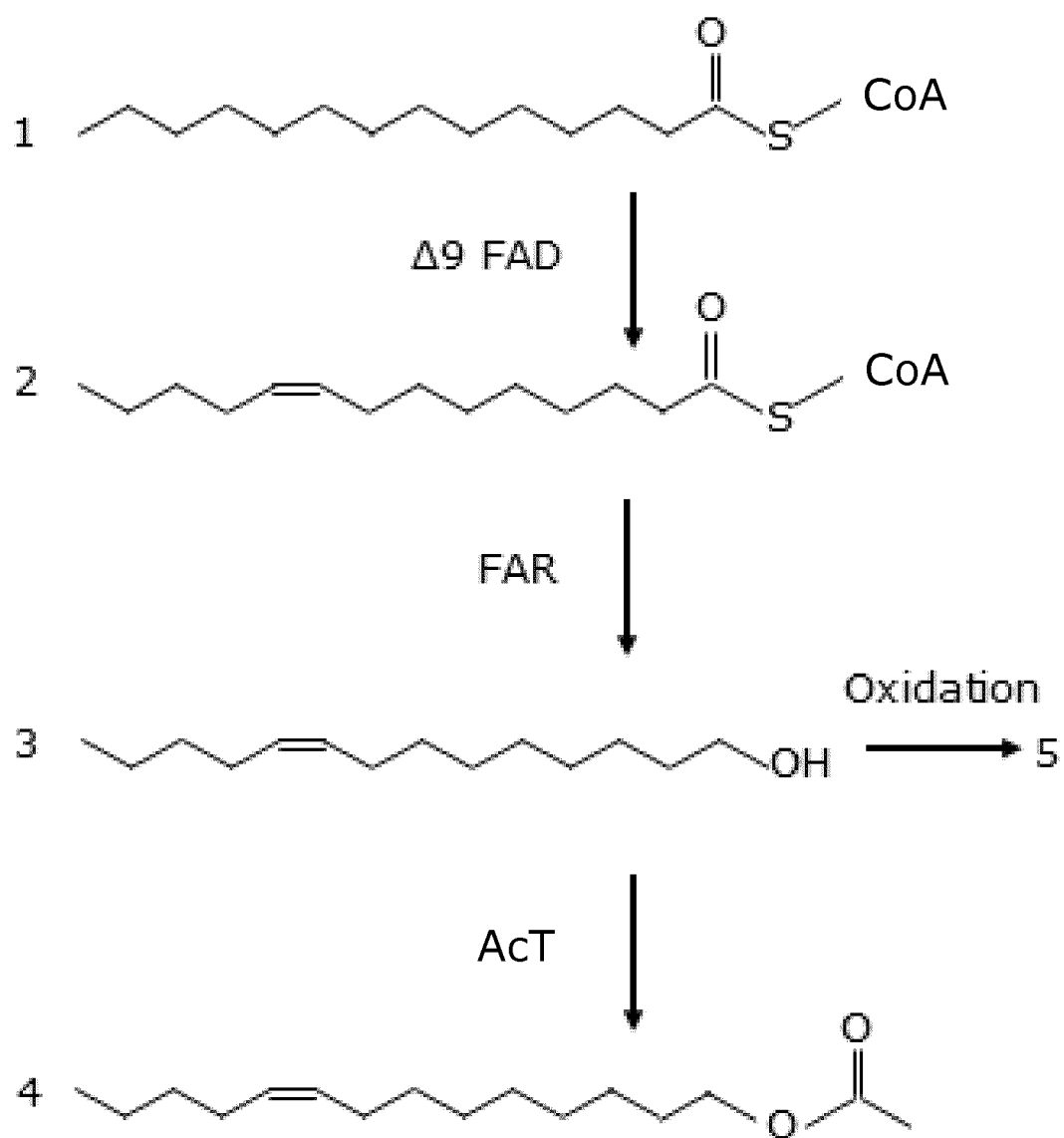


FIG. 2

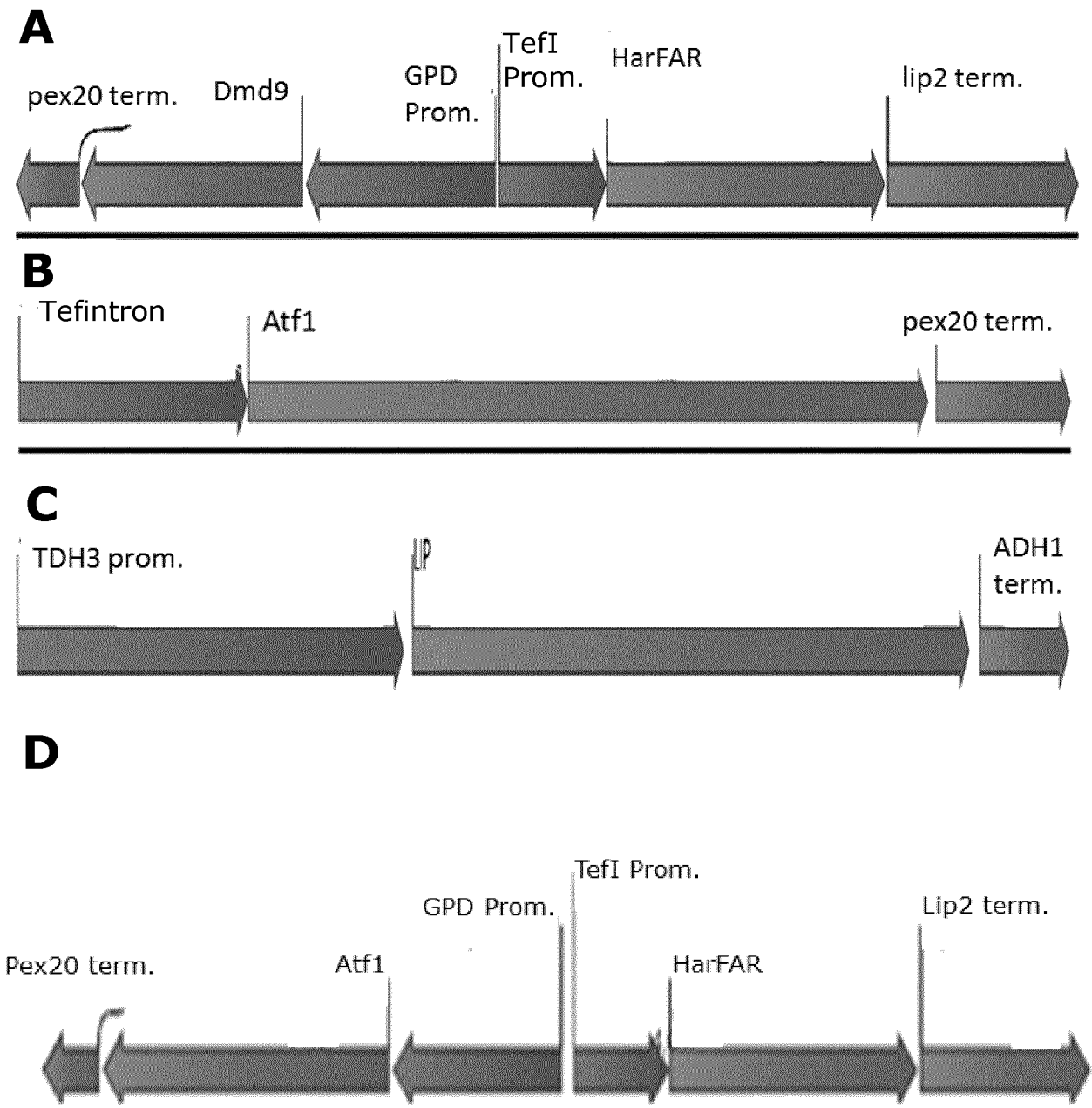


FIG.3 (CONT.)

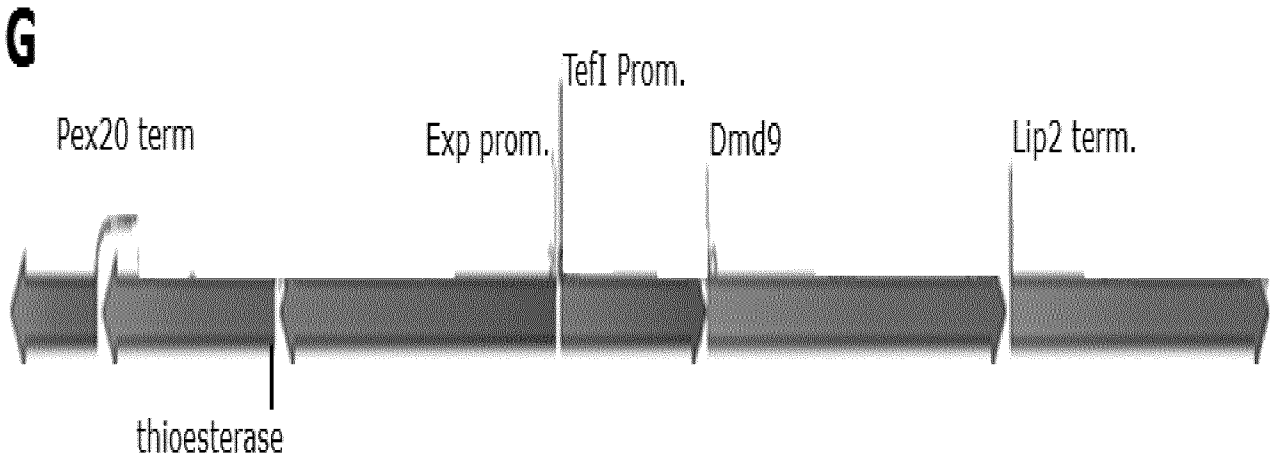
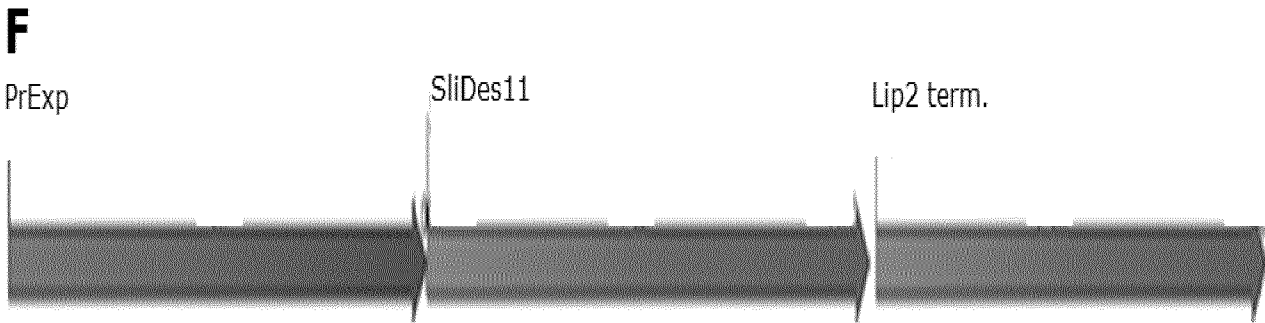
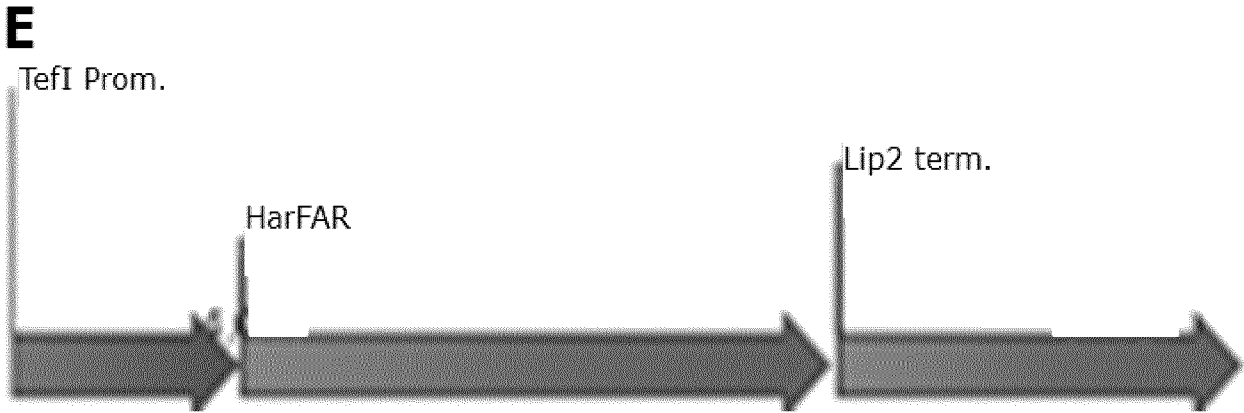
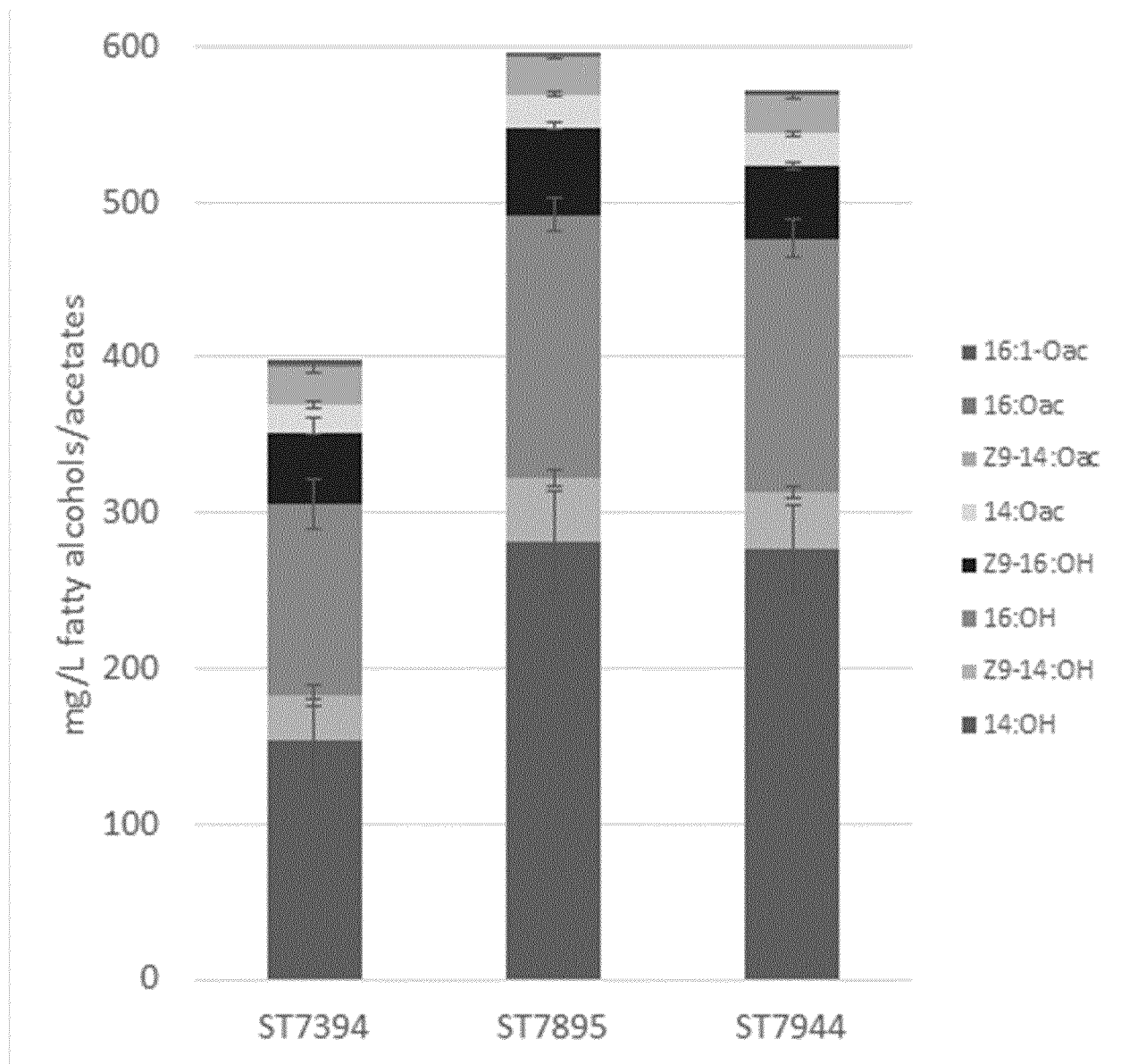


FIG. 3



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/083030A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/02 C12P7/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal , WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ÅSA K HAGSTRÖM ET AL: "A moth pheromone brewery: production of (Z) -11-hexadecenol by heterologous co-expression of two biosynthetic genes from a noctuid moth in a yeast cell factory" , MICROBIAL CELL FACTORIES, BIOMED CENTRAL, GB, vol . 12, no. 1, 13 December 2013 (2013-12-13) , page 125, XP021172484, ISSN : 1475-2859, DOI : 10. 1186/1475-2859-12-125 abstract page 6, column 1 ----- - / - -	17-19



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

28 February 2018

Date of mailing of the international search report

08/03/2018

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/083030

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DING BAO-JIAN ET AL: "The Yeast ATF1 Acetyl transferase Efficiently Acetylates Insect Pheromone Alcohols: Implications for the Biological Production of Moth Pheromones", LIPIDS, SPRINGER, DE, vol . 51, no. 4, 22 January 2016 (2016-01-22) , pages 469-475, XPQ35947504, ISSN: 0024-4201, DOI : 10.1007/S11745-016-4122-4 [retrieved on 2016-01-22] abstract page 470, column 1, paragraph 3 - column 2, paragraph 2</p>	1-19
A	<p>BAO-JIAN DING ET AL: "Analysis of the agrotis segetum pheromone gland transcriptome in the light of Sex pheromone biosynthesis", BMC GENOMICS, vol . 16, no. 1, 18 December 2015 (2015-12-18) , XP055379568, DOI : 10.1186/S12864-015-1909-2 abstract</p>	1-19